University of Alberta

Invasion history, population dynamics and biological control of *Profenusa thomsoni* (Konow) in Alaska

by

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A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
in
Forest Biology and Management

Department of Renewable Resources

Edmonton, Alberta
Spring 2008

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Your file Votre référence
ISBN: 978-0-494-45562-3
Our file Notre référence
ISBN: 978-0-494-45562-3

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Abstract

Profenusa thomsoni (Konow) has had a significant impact on urban birch (*Betula*) forests in Alaska. I investigated the invasion history of *P. thomsoni* in North America, analyzed mortality factors affecting the Alaskan population; and initiated a classical biological control program introducing *Lathrolestes luteolator* Gravenhorst from western Canada.

I observed no variation in mitochondrial DNA among *P. thomsoni* from North America and central Europe. This observation is consistent with one successful colonization event in North America but does not establish either direction of invasion or source of colonists. I compared Canadian and European populations of *Scolioneura betuleti* Klug with a related species, *Scolioneura vicina* Konow, to determine which species has been introduced to Canada. There was no difference in mtDNA between species, suggesting *S. vicina* and *S. betuleti* are the same species. The Canadian specimens of *Scolioneura*, therefore, could not be reliably identified as either of these species, although the phenology observed in Canada is more similar to that described for *S. vicina*.

I constructed egg and larval mortality schedules for *P. thomsoni* in Alaska to quantify resistance to leafminer population growth before introduction of a parasitoid. Survivorship was >40% for most larval instars, and <40% for eggs and late instars. The cause of >90% of larval mortality could not be associated to a specific cause. Therefore, I used classification-tree analyses to show that individual larval mortality was associated more with intraspecific competition and resource depletion than with host and abiotic factors. In addition to providing a baseline from which to evaluate the effectiveness of biological control agents, I demonstrated classification trees have practical use in the analysis of insect mortality.

In an attempt to control *P. thomsoni* I introduced 2685 (1155F:1530M) *L. luteolator* into Anchorage between 2004 and 2007. These parasitoids were collected from sites in northern Canada and transported to Alaska either as adults or pupae. Rearing success was poor (5-30% emergence) despite use of several different approaches. Nonetheless, adult females of *L. luteolator* were recovered in 2007, suggesting a population was established. I also evaluated rearing techniques for that may prove useful in future biological control efforts against *P. thomsoni* or other species.

For Amanda

Acknowledgements

I wish to thank my supervisors, Dr David Langor and Dr John Spence, and my committee, Dr Felix Sperling and Dr Victor Lieffers for their guidance, support and patience. I also wish to thank Dr Ed Holsten and Scott Digweed for their tireless support, encouragement and assistance. This project would not have been possible without them. I am also grateful for the generous support, enthusiasm, encouragement and assistance provided by Sandra Key and the staff of USDA Forest Service–Forest Health Protection-Alaska. I also thank the many members of the Spence and Sperling labs at the University of Alberta for much challenging discussion and for exposing me to different ways of looking at the world. I also thank my examining committee; Nadir Erbilgin, Andreas Haman and William Mattson for their thoughtful critique of this work.

Jerry Boughten, Richard Reardon and Roger Burnside provided invaluable technical, financial and administrative assistance over the course of this project. Ewald Altenhofer, Lorraine Nystrom, Lori Tucker, Daryl Williams, Marc Kenis and Mark Schultz all graciously gave of their time to collect specimens. I also thank Tommi Nyman for providing samples of *Scolionura* from Finland and Richard Laffin and Amanda Roe for invaluable technical support, training and patient discussion which greatly improved the work in Chapter 2. I am extremely and forever grateful for the assistance of Fionnuala and Maurya Braun, John Hard, James Kruse, Graham Mahal, Bryan Manly, Gwen Marcus, Steve Patterson, Michael Rasy, Corlene Rose, Cynthia Snyder, Anna Soper, Erin Ulroth, Roy van Driesche and Ken Zogas, all of whom contributed in no small way to this thesis.

This thesis would not have been possible without the financial assistance of the National Sciences and Engineering Research Council (PGS scholarship, operating grant to John Spence), the University of Alberta (Walter H Johns Scholarship), and the USDA Forest Service (Forest Health Protection grant to David Langor).

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Chapter 1. Introduction

Since humans began dispersing across the face of the earth, populations of associated animals and plants have been moved to new locations either by accident or design. While the introduction of one species by another to a new environment is not limited to human activity, humans are unique among organisms in the frequency, volume and speed with which we move organisms across the planet. Most of these introductions are unsuccessful or seemingly benign. However, some species find a habitat hospitable for establishment, persistence and spread (Elton 1958). These species are often referred to as 'invasive'. When an invasive species establishes, the ecological, economic and social impacts can be severe (Colautti *et al.* 2006; Pimentel *et al.* 2000). With increasing long-distance travel and global trade the rate of introductions of non-native or 'alien' species has increased (Brockerhoff *et al.* 2006). Although only a small percent of alien species cause significant economic damage (i.e., become invasive) or become a nuisance, all alien species should be considered potentially invasive. Thus, when an alien species is discovered in a new location there is almost immediate pressure to eradicate the introduced population. Alternately, when a species is discovered after it has become well established there is pressure to mitigate its effects. Eradication and mitigation can be expensive propositions costing billions of dollars. However one way to minimize these costs and decrease the risk of subsequent invasions is to understand the patterns and processes that drive alien species already present in new environments. If commonalities among different alien species can be identified then it may be possible to predict new invasions and minimize the effect of aliens.

Four important pieces of information are essential to deal with a new invasion: identity of the species, origin or native range; the extent of its range in the invaded territory (i.e., geographic and host distributions); and an assessment of its damage and potential impact. The identity and origin of an alien species allows access to the global cache of information about that species. Such information helps with assessment of potential risks to the economy and environment (Armstrong and Ball 2005), implementation of measures to slow or prevent further invasions (Baker *et al.* 2005), and elucidation of modes of entry (Walters *et al.* 2006). Information about the geographic and host range of the species in the invaded territory is useful for evaluating the potential risk for spread, either to new hosts or to new locations, whereas information about the damage potential of the alien species in the new range requires knowledge of its population dynamics and how it interacts with its environment. Most alien species become disassociated from their natural enemies and competitors upon introduction, and as a result introduced populations can grow to be much larger than those that occur in the native home range (Elton 1958; Niemela and Mattson 1996; Mattson *et al.* 2008). Thus, in order to understand the processes regulating population dynamics in the new range it is important to understand how alien populations respond to mortality agents in the new environment. Lastly, once it is known where a species comes from and how it behaves, it is possible to identify factors that can control it if the species becomes the cause of serious economic or aesthetic damage. Collectively this sort of applied work on alien species requires focus at multiple scales using different techniques specific to the goals of understanding alien populations as they interact with a new environment.

Study system

The ambermarked birch leafminer *Profenusa thomsoni* (Hymenoptera: Tenthredinidae: Heterarthrinae) (Konow) is a sawfly native to the Palearctic ranging from Great Britain to Turkey and Japan. All 13 species in the genus *Profenusa* are leafminers but only *P. thomsoni* attacks birch (*Betula* L.). In Europe and Asia, *P. thomsoni* attacks *B. dahurica* Pall.; *B. mandshurica* var. *japonica* (Miq.) Rehder; *B. pendula* Roth; *B. pubescens* Ehrh.; and *B. turkestanica* Litv.; in North America *B. alleghaniensis* Britton; *B. glandulosa* Michx.; *B. nana* L.; *B. neoalaskana* Sarg.; *B. occidentalis* Hook.; *B. papyrifera*

Marsh.; and *B. populifolia* Marsh. are recorded as hosts (Cheng and LeRoux 1965; Digweed *et al.* 1997; Martin 1960; Peirson 1929; Pieronek and Soltyk 1993; Schönrogge and Altenhofer 1992; Species names follow Govaerts and Bopp 1998). *Profenusa thomsoni* occasionally co-occurs with other members of the Heterarthrinae that also mine birch: *Fenusa pumila* Leach; *Fenusella nana* (Klug); *Heterarthrus nemoratus* (Fallén); *Scolioneura vicina* Konow; and *S. betuleti* (Klug). All of these species have been inadvertently introduced to North America, with the exception of *S. betuleti* (Chapter 2).

Profenusa thomsoni is univoltine, overwintering as an eonymph (a late instar, quiescent larval stage) inside a pupal cell it constructs underground below the tree it developed upon. Emergence occurs between early June and mid-July depending on latitude (Digweed 1998; Martin 1960; Pschorn-Walker and Altenhofer 1989; Schönrogge and Altenhofer 1992; Snyder *et al.* 2007), and in some locations adults can still be found as late as mid-August. No males are recorded and thus the species is assumed to be parthenogenic (Benson 1952, 1959; Pschorn-Walker and Altenhofer 1989). Females begin laying eggs one to two days after emerging from the soil, reaching maximum egg output four to five days after emergence (pers. obs.). The average lifespan is 7 days (pers. obs.).

Eggs are laid individually in small slits cut in the upper epidermis of birch leaves and hatch within a week to twelve days. Each of the first five larval stages are adapted for feeding within the leaf, being dorso-ventrally flattened and prognathous, however the sixth instar, adapted for movement to overwintering sites, retains the typical sawfly form (not flattened and hypognathous). Younger larvae in leaves that have been defoliated will also attempt to disperse but they are incapable of entering a new leaf and eventually perish (Digweed 1998; Martin 1960). Larval development takes approximately 24 days (Martin 1960) after which newly molted sixth instar larvae emerge from holes they cut in the top or bottom of the leaf. The larvae then drop to the ground and enter the soil where they web together soil particles and organic material to construct their own pupal cell. Within its pupal cells each larva enters an eonymph stage and adopts a characteristic 'Sheppard's crook' shape, with the head curved forwards and the end of the abdomen curved dorsally. This transformation appears to not involve a molt as no shed exoskeleton is found inside the pupal cell. Larvae overwinter in this form and then molt to exarate pupae shortly before emergence the following year. Some individuals exhibit extended diapause, emerging as adults after a second winter spent as an eonymph in the pupal cell (pers. obs.; S Digweed pers. comm.)

Infestations of *P. thomsoni* can be severe and large populations can defoliate whole trees. Larvae consume the upper mesophyll layer of their host leaf resulting in a blotch shaped mine on the upper surface. Under moderate-to-high densities mines coalesce and multiple larvae can be found feeding in the same mine. When densities are high, damage is obvious and fully defoliated leaves turn brown. As most outbreaks occur in urban forests the major public concern is the loss in aesthetic value of individual trees. However, repeated years of defoliation may decrease a tree's ability to resist other insects or diseases (Hoch *et al.* 2000) and, while no direct effect on tree health has been demonstrated for *P. thomsoni*, an outbreak of *H. nemoratus* was associated with a decrease in annual increment of birch trees in Maine during the 1920s (Pierson and Brower 1929).

Outbreaks of *P. thomsoni* have been recorded in North America in Ontario (ON) (Martin 1960), Wisconsin (WI) (Benson 1959), Alberta (AB) (Drouin and Wong 1984), the Northwest Territories (NT) (Digweed and Langor 2004), British Columbia (BC) (pers. obs.) and Alaska (AK) (Snyder *et al.* 2007) as well as in Poland (Pieronek 1995; Pieronek and Soltyk 1993). However, in Europe outbreaks are rare and individuals can be difficult to find (Fisher 1997; Schönrogge and Altenhofer 1992). The duration of outbreaks in North America varies with the longest lasting from the early 1970s through the mid-1990s in AB. All outbreaks recorded in the literature have occurred in urban forests, with the exception of an outbreak north of Sault Ste Marie, ON (Martin 1960). The leafminer can be found in native birch forests across North America (e.g. Digweed 1998) but with rare exceptions (e.g. Snyder *et al.* 2007), populations in such forests never reach the large size of those typical of many urban forests. In general leafminers are suppressed by parasitoids (Connor and Taverner 1997; Hespenheide 1991), however, both in Europe and North America, *P. thomsoni* has a much less diverse parasitoid fauna than do the other species of birch leafminers (Digweed *et al.* submitted; Schönrogge and Altenhofer 1992), which may partially explain the large populations seen in urban forests. In North America there is a species of koinobiont endoparasitoid, *Lathrolestes luteolator*[1] Gravenhorst (Hymenoptera: Ichneumonidae) that was responsible for suppressing populations in AB in the mid-1990s (Barron 1994; Digweed 1998; Digweed *et al.* 2003). The parasitoid suppresses *P. thomsoni* by attacking the leafminers larval stage as it feeds within leaves and consumes the leafminer's pre-pupal stage after it overwinters underground. Female *L. luteolator* initiate an attack by laying an egg within a *P. thomsoni* larva, this egg hatches soon after but the resulting larva stays dormant until its host has entered its pupal cell, at which time it begins feeding. In addition to this parasitoid, other control options, such as stem-injected and soil-drench systemic pesticides, exist and are commonly prescribed (Drouin and Wong 1984; Marion *et al.* 1990) but involve environmental risk and are often expensive or difficult to apply. Furthermore, their effectiveness can vary even when applied correctly (pers. obs).

Profenusa thomsoni has moved across North America in a short time span since it was first discovered in Connecticut in 1923 (Smith 1971). By 1950s it was recorded from the Northeast United States west to Illinois and WI (Benson 1959; Ross 1951). Canadian records date to 1948 from Sault Ste Marie, ON (Canadian National Collection of Insects specimen Hymen #05-130 Martin 1960), but the species was likely present well before that time as it was often misidentified as *F. pumila* (Martin 1960). Museum specimens exist from Fort Gary, Manitoba in 1950 (CNCI specimen Hymen #05-114). Drouin and Wong (1984) state that *P. thomsoni* damage was abundant in Alberta by at least 1972, however, Smith (1971) did not report any specimens from Saskatchewan or AB in his publication about Nearctic sawflies from the same period. By 1994 *P. thomsoni* had reached Yellowknife, NT and by 2003 was found in Dawson City, Yukon Territory (Digweed and Langor 2004; Digweed *et al.* 1997). *Profenusa thomsoni* appeared in southeast AK sometime prior to 1988 (USDA Forest Service 1991). In the early to mid 1990s, the species was introduced to Anchorage, AK, although as was the case in the 1940s in Canada, it was misidentified for a period as *F. pumila* (Snyder *et al.* 2007). When the work for this thesis began in 2003, AK populations of *P. thomsoni* were believed to be restricted to Haines, Anchorage and one site on Eielson Air Force base near Fairbanks. However, road surveys made between 2003 and 2005 established that the species was actually widespread outside of Anchorage through the Matanuska-Susitna Valley and portions of the Kenai Peninsula (Snyder *et al.* 2007). Other populations were later found in Fairbanks extending west along the George Parks Highway c. 50 Km to the popular tourist stop, “Skinny Dick’s Halfway Inn” outside of Ester, AK. The research presented herein was conducted on these Alaskan populations.

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[1] A recent taxonomic study suggests this species may not be *Lathrolestes luteolator* (A Bennett, Canadian National Collection of Insects, pers. comm.). However, I use the *L. luteolator* designation in this thesis as it is the current published name for the species attacking *Profenusa thomsoni* (Barron 1994)

In this thesis I evaluate the impact of *P. thomsoni* in relatively newly colonized forests in Alaska. Using these populations of *P. thomsoni* as a model system to understand alien invasive defoliators, I ask three basic questions:

- Where did *P. thomsoni* in Alaska and North America originate from?
- What patterns and causes of mortality influence this species in its new environment?
- Can a biological control agent, *L. luteolator*, be established to achieve suppression of outbreak populations?

In Chapter 2, I address the first question by reconstructing the invasion history of *P. thomsoni* in North America using mitochondrial DNA sequence data to investigate the possible European source population of *P. thomsoni* and estimate the number of separate introductions into North America. In Chapter 2, I also explore the use of molecular tools to distinguish between *S. vicina* and *S. betuleti*, two morphologically indistinct species of birch leafminer, one of which is a recent introduction to North America. In Chapter 3, I describe the effects of intraspecific competition, host-tree, and abiotic factors associated with *P. thomsoni* egg and larval mortality and then relate these factors to the survivorship, developmental time and population size of each instar. Finally, in Chapter 4, I describe the introduction of a biological control agent to suppress *P. thomsoni* in Alaska, summarize the early results, and discuss the potential for successful introduction by examining previous studies. In Chapter 5, I discuss the implications of these studies and consider the role of using multiple techniques to understand and mitigate the effects of alien species in new landscapes.

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Chapter 2. Mitochondrial DNA variation in two invasive birch leafmining sawflies in North America.[1]

Introduction

The origins and impacts of alien species are of increasing interest (Pimentel *et al.* 2000; Sakai *et al.* 2001; Simberloff *et al.* 2005). Knowing the identity and home range of an alien species is necessary to obtaining access to the global cache of information about that species. Such information helps with: assessment of potential risks to the economy and environment (Armstrong and Ball 2005), implementation of measures to slow or prevent further invasions (Baker *et al.* 2005), and elucidation of modes of entry (Walters *et al.* 2006). Genetic tools can be accurately and reliably applied to investigate species identity and origin. Many studies have used molecular tools to examine species identity, invasion routes, host range and genetic diversity of alien arthropods (e.g., Cognato *et al.* 2005; Davies *et al.* 1999; Navia *et al.* 2005) however few studies have applied genetic techniques to the study of alien Hymenoptera (Baker *et al.* 2003; Hufbauer *et al.* 2004; Johnson and Starks 2004; Tsutsui *et al.* 2000), and none have examined introduced sawflies (Symphyta).

Profenusa thomsoni (Konow 1886) and *Scolioneura betuleti* (Klug 1816) (Hymenoptera: Tenthredinidae) are two birch leaf mining sawflies that are native to Europe but were accidentally introduced to North America. These species are rare or occasionally minor pests of birch (*Betula* L.) in Europe (Kenis and Carl 1995; Pieronek 1995; Schönrogge and Altenhofer 1992) but in North America have become significant pests of birch (Benson 1959; Digweed *et al.* 2003; Nystrom and Evans 1989). The larvae of both species are internal leaf feeders, damaging the photosynthetic layer and resulting in both aesthetic damage to trees and a loss of tree vigor, potentially causing death.

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[1] *A version of this chapter has been published. MacQuarrie CJK, Langor DW, Sperling FAH 2007. The Canadian Entomologist. 139:545-553*

Profenusa thomsoni was first recorded in North America from the eastern United States in 1923 (Ross 1951) and from Canada in central Ontario (ON) in 1955 (Lindquist 1955). However, *P. thomsoni* was likely well established in Canada before 1955 as prior to that time it was commonly confused with another birch leafmining sawfly, *Fenusa pumila* Leach 1817 (Hymenoptera: Tenthredinidae) (Martin 1960) and adults had been collected in Fort Gary, Manitoba and Sault Ste Marie, ON in 1948 and 1950, respectively (Canadian National Collection of Insects specimens Hymen #05-114 and Hymen #05-130). *Profenusa thomsoni* became a significant pest in eastern Canada by the late 1950's (Martin 1960), in Alberta (AB) by the 1970's (Drouin and Wong 1984) and in Alaska (AK) by the 1990's (Snyder *et al.* 2007). The species is univoltine and likely parthenogenic as no males are recorded (Benson 1959). Females emerge in late spring to early summer, and lay eggs singly or in clusters on fully expanded birch leaves. Larvae complete development by early fall and final instars emerge and fall to the ground where they construct earthen cells and overwinter as prepupae (Chapter 1; Martin 1960).

Records of *P. thomsoni* (Benson 1959; Lindquist 1955) suggest the species was introduced somewhere in eastern North America, and likely much earlier than it was first recorded (i.e., 1923). Given that most sawflies are weak flyers (Benson 1950), and that *P. thomsoni* has only one generation per year (Martin 1960), it is unlikely that it was able to spread from ON to AK in less than 60–80 years solely under its own power. As birches are popular horticulture species it is reasonable to infer that some introductions were associated with commercial transfer of infested plants in the horticultural trade. Thus new populations are likely established by a small number of founder individuals, creating genetic bottlenecks from which it may be possible to identify the invasion history of the species.

Scolioneura betuleti is a more recent import to North America, first recorded from one site north of Toronto, ON in 1983 (Evans *et al.* 1985) It has since expanded its range within ON and outside the province east to Prince Edward Island and Newfoundland and Labrador (NL) and west to British Columbia (BC) (Digweed *et al.* in prep). Nystrom and Evans (1989) identified the species in Canada as *S. betuleti* using keys available at the time. Later, Altenhofer and Taeger (1998) split *S. betuleti* in Europe into *S. betuleti* and *Scolioneura vicina* Konow 1894 (Hymenoptera: Tenthredinidae), basing their decision on adult phenology and host plant preference (the species are morphologically indistinct as both adults and larvae). *S. vicina* is described as a spring-flyer, feeding only on *Betula pubescens* Ehrh. and *B. pendula* Roth, while *S. betuleti* flies in the fall and feeds on the same *Betula* species, but also *Alnus viridis* (Chaix) DC (Betulaceae). Thus according to Altenhofer and Taeger (1998) the species found in Canada is more likely to be *S. vicina* than *S. betuleti*, based on flight phenology. While *S. betuleti* and S. *vicina* are not currently considered pests of *Betula* in Canada or Europe there is a need to resolve this taxonomic issue as a prerequisite to future work on this species.

This chapter aims to reconstruct the invasion history of *P. thomsoni* in North America and resolve the taxonomy of *Scolioneura* Konow 1890 (Hymenoptera: Tenthredinidae) species in Canada and Europe using mitochondrial DNA (mtDNA) sequence data. I used an 840 base-pair (bp) region of the mitochondrial genome of *P. thomsoni* and a 716 bp region from both putative *Scolioneura* species to assess: 1) the source population in Europe for *P. thomsoni* and the number of separate introductions into North America; and 2) which of two putative birch-inhabiting *Scolioneura* species recognized by Altenhofer and Tager (1998) occurs in Canada. I selected mtDNA as my marker because sequence data are relatively easy to obtain and this genome has a high rate of evolution (Simon *et al.* 1994).

Methods

Specimen Collection

Profenusa thomsoni larvae were extracted from leaves of *Betula* collected from ten North American sites and two central European sites (Table 2.1) and preserved in 80–100% ethanol. Identification at the time of collection was based on host plant, larval morphology and mine shape (Lindquist 1959), and were later confirmed by me. Attempts were made to collect throughout the range of *P. thomsoni*, with a few gaps in coverage because of unavailability of *P. thomsoni* populations or collectors.

Scolioneura larvae were collected in the same manner as *P. thomsoni* at one site in eastern Austria and another in ON, Canada (Table 2.1). All European *Scolioneura* specimens were identified to species by Ewald Altenhofer at the time of collection (Table 2.1) and their phenology corresponds to that given for these species in Altenhofer and Taeger (1998) (Table 2.1). All Canadian material was identified by the collector and confirmed by me using descriptions and figures from Nystrom and Evans (1989). Collectors initially stored larvae in ethanol at room temperature or -20°C until they were shipped. After arrival at the laboratory, specimens were stored at -20°C.

DNA Extraction, Amplification and Sequencing

Mitochondrial DNA from *P. thomsoni* and *Scolioneura* species was extracted using a QiaAmp DNA minikit (Qiagen, Mississauga, ON) using the manufacturer's recommended protocol. Extractions made from the larval abdomen provided sufficient genetic material for amplification by polymerase chain reaction (PCR). The larval head and thorax for each sequenced specimen were stored in 100% ethanol at -70°C and deposited as vouchers, along with a selection of intact larvae from the same collection locality and date, in the Strickland Entomological Museum at the University of Alberta, in Edmonton, AB, Canada.

A fragment spanning the *cytochrome oxidase I* (COI), *tRNA*^{LEU} and *cytochrome oxidase II* (COII) region of the mitochondrial genome was amplified from *P. thomsoni* using four sets of heterologous primer pairs (Table 2.2). From this fragment I obtained 840 bp of sequence for a minimum of two specimens from all sites and an additional 1318 bp of sequence from one specimen from the most geographically separate sites, giving a total sequence of 2158 bp (Table 2.1). Two to five specimens per site were sequenced. I observed no sequence variation between any of the 32 specimens, regardless of collection site, therefore additional sequencing to assess haplotype frequencies was not necessary. A representative sequence of either 840 or 2158 bp from each site was deposited in GenBank (840 bp: ACC#EF445947–EF445951, 2158 bp: ACC#EF445956–EF445961).

A fragment of the COI gene was amplified from all *Scolioneura* specimens using primers Jerry & Pthom1 (Set C, Table 2.2) from which 716 bp of sequence (GenBank ACC#EF445952–EF445955) was obtained. PCR amplification and sequencing reactions were carried out using standard laboratory methods, exact details of which are presented elsewhere (Abe *et al.* 2005; Laffin *et al.* 2005; Roe *et al.* 2006). I also used additional sequence data obtained from GenBank in my analysis, specifically 349 bp derived from *S. betuleti* from northern Finland (GenBank accession number ACC#DQ302242) (Nyman *et al.* 2006). The differences in nucleotide sequence length between the GenBank sequence and my sequences were coded as missing data. I also acquired a specimen from the same collection date and location from Tommi Nyman (Department of Biology, University of Oulu, Oulu, Finland) and confirmed its identity.

Analysis

All *P. thomsoni* and *Scolioneura* sequences were aligned in Sequencher (ver. 4.1, Gene Codes Corporation, Ann Arbor, MI 2001) using default settings, with ambiguous base calls corrected by eye; data derived from primer regions were removed prior to alignment. Contiguous strands (contigs) were assembled by aligning fragments generated from primer pairs, then aligning contigs from overlapping regions. Consensus strands were then generated from bidirectional contigs or >1 uni-directional strands for the same region generated from independent sequencing reactions (with exceptions: 338 bp in one Anchorage, AK specimen; no reverse complement between RonII & K525 for one Eielson Air Force Base, AK specimen and one Prince George, BC specimen). Base frequencies were calculated using PAUP* 4.4 (Swofford 2003). Alignment to *Drosophila yakuba* Burla (Diptera: Drosophilidae) (Clary and Wolstenholme 1985) was done by eye. Divergences between the *S. betuleti* sequence from GenBank and those obtained in this study were examined visually using MacClade 4.05 (Maddison and Maddison 2002) to evaluate the position of amino acid base pair changes. Analysis of the *P. thomsoni* data set was done by eye.

Results

Profenusa thomsoni

I obtained sequences from 32 *P. thomsoni* specimens. The 2158 bp sequence (n = 12) corresponded to positions 1461–3564 of the *D. yakuba* sequence. Inserts in the *P. thomsoni* sequence were observed at the following positions relative to *D yakuba*: 39 bp at position 1474, 15 bp at 3009 and 3 bp at 3062. The 840 bp (n = 32) sequence was aligned to positions 1736–2575 of *D. yakuba* with no insertions or deletions (indels). There was no variation among specimens in either the 840 bp or 2158 bp sequence.

Scolioneura

I obtained 716 bp of sequence from nine specimens of European *S. vicina*, six of European *S. betuleti* and two of Canadian *Scolioneura*. This sequence corresponds to positions 2209–2925 of *D. yakuba*, with no indels. Additional Canadian material was collected and mtDNA extracted but its DNA could not be amplified despite repeated attempts. I identified three haplotypes: haplotype A was observed in all nine European *S. vicina*, two of six European *S. betuleti* specimens, and both Canadian specimens; haplotype B was observed only in four European *S betuleti* specimens; and haplotype C was observed only in sequence obtained from GenBank. Genetic divergence between central European haplotypes A and B was 0.14%, or one nucleotide. Genetic divergence between central European haplotypes (A + B) and the northern Finland haplotype (C) was 2.08%. The inferred amino acid sequence was the same for all haplotypes.

Discussion

To my knowledge this study is the first to use genetic methods to attempt to resolve the invasion history of an alien sawfly species and to resolve a question of sawfly species identity. Furthermore, very few studies have used genetic characters to resolve the taxonomy of groups within the Symphyta (Heidemaa 2004; Nyman *et al.* 2006; Schulmeister 2003) and other attempts to re-construct the invasion history of parthenogenic insects have been limited to the Hemiptera (e.g., Downie 2002; Gwiazdowski *et al.* 2006; Havill *et al.* 2006).

Genetic variation in *P. thomsoni*

I observed no variation among *P. thomsoni* individuals from North America and Europe. This observation is consistent with a scenario that there was one successful colonization event in North America, although that interpretation is preliminary in the context of the limited sampling in my study, especially in Europe. I do not know the full range of the single haplotype - other than it occurs at one locality in each of Switzerland and Austria – but the introduction to North America must have come from within the presumably European range of that haplotype. If a greater amount of genetic variation occurs in Europe, then it would be plausible that the original colonists to North America were relatively few and genetically uniform. In any case, these data are not consistent with the hypothesis of Schönrogge and Altenhofer (1992) that *P. thomsoni* is a native North American species, based on the presence of the specialist parasitoid *Lathrolestes luteolator*[2] Gravenhorst (Hymenoptera: Ichneumonidae) only attacking the leafminer on that continent. It is highly unlikely, if *P. thomsoni* were native to both Europe and North America, that there would be no genetic differentiation between populations from the two continents.

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[2] See footnote to Chapter 1 on page 5.

The lack of genetic divergence between the two sampled populations of *P. thomsoni* in its native Europe was unexpected, especially as I observed intra-population differences in *Scolioneura*. Further sampling over a broader geographic range may reveal the presence of other European haplotypes. This has been shown in other sawfly species over a similar geographic range using allozymes (Muller *et al.* 2004) and in the present study where I observed two haplotypes over the same sampled range for *Scolioneura*. It is possible that I did not sample a sufficient number of individuals in Europe (n = 8) to detect the presence of rare haplotypes, although if these haplotypes did exist and comprised a significant portion of the population I should have detected some within my North American specimens (n = 24). Alternately, there exist multiple processes that could have caused a homogenization of mtDNA diversity of *P. thomsoni* prior to its introduction into North America (Hurst and Jiggins 2005). For example, selective sweeps by endosymbionts such as *Wolbachia* have been suggested to cause low mtDNA diversity in other species (Hurst and Jiggins 2005) and parthenogenesis in haplo-diploid groups (Werren 1997). If the mitochondrial genome I sequenced is representative of total genetic diversity, my data suggest *P. thomsoni* may have a reduced adaptive capacity to deal with strong selective forces such as natural enemies, diseases, or abiotic factors that could be used for biological control.

Scolioneura genetic variation and taxonomy

The mtDNA sequences of putatively distinct *S. betuleti* and *S. vicina* are genetically similar, differing only 0.14% between haplotypes A and B. It is possible that more variation occurs throughout the range of *S. betuleti*/*S. vicina* as suggested by the 2.08% variation between haplotypes from central Europe (haplotypes A & B) and northern Finland (haplotype C). However, this amount of variation is somewhat ambiguous in determining if the *S. betuleti* specimen from northern Finland is part of a distinct species as two percent variation has been suggested as a cut – off for species delimitation using mitochondrial DNA sequence data (Hebert *et al.* 2003 but see Cognato 2006).

The presence of only haplotype A in Canadian *Scolioneura* does not allow a clear genetic diagnosis of the Canadian material, as Haplotype A is present in both putative European *Scolioneura* species (Table 2.1). In the absence of definitive genetic data, the phenology of the Canadian population (i.e., spring–flying adults) leads to the conclusion that this population is *S. vicina*, not *S. betuleti* as reported (Nystrom and Evans 1989). However it is necessary to re-examine whether *S. vicina* is a valid species; Altenhofer and Taeger (1998) resurrected the name from synonymy on the strength of biological and host preference data. My molecular data suggest that *S. betuleti* and *S. vicina* are possibly conspecific, and thus I suggest that *S. vicina* could be considered a junior synonym of *S. betuleti* if these data can be supported by other molecular markers.

The observation that only fall-flying *S. betuleti* will utilize *A. viridis* (Altenhofer and Taeger 1998) in Europe should be examined further to understand why spring-feeding *S. vicina* do not utilize this host. It is possible that newly expanded *A. viridis* leaves are not accepted for oviposition by spring-flying *S. vicina*, but as leaves age, changes occur such that *Alnus* becomes an acceptable host, for fall-flying females. This could be tested by altering diapause cues in reared specimens so that spring- and fall-flying *Scolioneura* adults could be exposed to 'fall' *Alnus* foliage and *vice versa*.

Further avenues of investigation are provided by the finding that *Scolioneura* larval skins were recently recovered from sites in NL and BC (Digweed *et al.* submitted). It would be useful to sample these populations and any intermediates to evaluate the invasion history of *S. vicina* in Canada. Furthermore, surveys in

Europe and Canada to delineate the ranges of mtDNA haplotypes may suggest where to search for new natural enemies to introduce against *S. vicina* in North America.

Application to management of birch leafminers

The genetic techniques developed in here have practical application in the management of future birch leafminer outbreaks. Using these techniques It should now be possible to conduct similar studies of the other, alien birch leafmining species known from North America (*Fenusa pumila* Leach; *Fenusella nana* [Klug]; *Heterarthrus nemoratus* [Fallén]; Hymenoptera: Tenthredinidae) to determine their home ranges. This information would be immediately applicable to guide a search in the Palearctic for potential natural enemies of *F. nana* and *S. vicina*. These two leafminer species are widespread in Canada and have the potential to cause significant damage but they have not yet been the target of biological control introductions (Digweed *et al.* submitted). Knowing the home range of these species would allow biocontrol practitioners to target specific regions as sources of natural enemies (i.e., parasitoids). It is likely that natural enemies from the home range of a species are better adapted to exploit their normal host (Gwiazdowski *et al.* 2006) and thus contribute to the control of exotic populations. Likewise, investigations in the home range of all five leafminer species might reveal resistant birches that could be used in breeding programs to produce leafminer resistant cultivars for use in urban plantings (Chapter 3; *sensu* Fiori 1987; Fiori and Dolan 1984; Hoch *et al.* 2000).

Comparison of genetic diversity within and among populations of the five species of Palearctic birch leafmining sawflies in North America could give insight into patterns of genetic diversity associated with successful colonization and range expansion. Repeated patterns among closely-related invasive species that have colonized the same area would be interesting and perhaps reveal general underlying processes. As these five species share many life history traits (e.g., host plant, larval ecology, etc) and a similar residence time in North America but differ in other traits (e.g., mating strategy, generation time) it may be possible to determine relationships among patterns of genetic diversity, invasion histories and life history traits associated with successful introductions (Lambrinos 2004). For instance, because *P. thomsoni* is parthenogenic it should require fewer introductions to establish a population and the resulting populations should have a lower level of genetic diversity than sexually reproducing *F. pumila* (Memmott *et al*. 2005; Travis *et al*. 2005).By extending these studies to other Palearctic Tenthredinid sawflies that have not colonized North America it might also be possible to identifying traits associated with 'invasiveness' that could prove valuable in predicting future invasions and managing existing invasions.

Note added after publication

After the publication of this chapter (MacQuarrie *et al*. 2007), Linnen and Farrell (2007) published a study that suggests an alternate interpretation of the *Scolioneura* data. Those authors demonstrated multiple polyphyletic species and disagreement between the mitochondrial and nuclear phylogenies for the *leconti* group of the North American clade of *Neodiprion* Rohwer (Hymenoptera: Diprionidae). This evidence suggests extensive recent and ancient hybridization events had occurred within that group of pine-feeding sawflies. If similar hybridization events occurred in *Scolioneura* then *S. vicina* and *S. betuleti* could indeed be distinct species. If so, the lack of differentiation between the central European specimens was the result of sampling the same mitochondrial haplotype in species that have hybridized. Furthermore, the 2.08% difference in sequence between central Europe (haplotypes A & B) and northern Finland (haplotype C) specimens could also represent hybridizations and not large sequence differences between widely separated populations. Further sampling of both *S. vicina* and *S. betuleti* populations throughout the Palearctic and the invaded range in the Nearctic, and sampling of other related species within the Tenthredinidae, would be required to determine if hybridizations have occurred.

Table 2.1 *Profenusa thomsoni* and *Scolioneura* sp. collection and sequencing information. Uppercase letters denote haplotypes of *Scolioneura* in the "no. specimens sequenced" column; see text for details.

Collection site	Lat.	Long.	Date	Collector	no. specimens sequenced*
Profenusa thomsoni					
USA, AK, Anchorage	61.169	-149.918	27 vii–25 viii 2005	C. MacQuarrie	$2^{a,b}$
USA, AK, Eielson Air Force Base	64.689	- 147.079	15 vii 2004	C. MacQuarrie	2^{a}
USA, AK, Haines	59.150	- 135.320	24 viii 2004	M. Schultz	2^{a}
CAN, BC, Prince George,	53.550	- 122.450	28 viii 2005	C. MacQuarrie	3^{a}
CAN, NWT, Yellowknife,	62.272	- 114.210	2 viii 2005	S. Digweed	3^{a}
CAN, SK, La Ronge,	55.070	- 105.324	28 viii 2005	S. Digweed	$3^{a,b}$
CAN, MB, Swan River	52.062	- 101.160	2 ix 2005	S. Digweed	3^{a}
CAN, ON, Wawa	47.590	- 84.470	4,8 viii 2005	K. Nystrom	$3^{a,b}$
CAN, NS, Dartmouth	44.476	- 63.352	23 vii 2005	D. Williams	$3^{a,b}$
Austria, Lower Austria, Arbesbach	48.483	14.950	7 viii 2005	M. Kenis	$5^{a,b}$
Switzerland, Neuchâtel, Les Ponts-de-Martel	47.000	6.733	6 viii 2005	M. Kenis	$3^{a,b}$
Scolioneura					
Austria, Lower Austria, Etzen	48.566	15.033	17 vi 2005	E. Altenhofer	$9A^{c}$
CAN, ON, Brantford	43.900	- 80.140	23 vi 2005	L. Tucker	$2A^{c}$
Austria, Lower Austria, Etzen	48.566	15.033	10–28 ix 2005	E. Altenhofer	$2A^{c}$, $4B^{c}$
Finland, Kilpisjärvi	69.046	20.795	10.viii.2001	T. Nyman/V. Vikberg	$1C^{d}$

*All specimens were collected as mid- to late-instar larvae from *Betula* sp. All *Profenusa thomsoni* specimens and *Scolioneura* specimens from Austria and Canada are deposited at the University of Alberta, Strickland Museum, Edmonton, Alberta, Canada. *Scolioneura* specimens from Finland are deposited at the Zoological Museum of the University of Oulu, Oulu, Finland.

[a] 840 bp sequence from all specimens, GenBank ACC#EF445947–EF445951

[b] 2158 bp sequence from one specimen, GenBank ACC#EF445956–EF445961

[c] 716 bp sequence from all specimens, GenBank ACC#EF445952–EF445955

[d] 349 bp from GenBank ACC#DQ302242 (Nyman *et al.* 2006)

Table 2.2 Mitochondrial DNA primers used for *Profenusa thomsoni* and *Scolioneura* sp. COI – *tRNA*^{LEU} – COII amplification. Direction and location of primers follows Simon *et al.* 1994.

Primer Set	Primer	Direction and Location	Sequence (5' – 3')
A	K698[a]	TY-J-1460a	TACAATCTATCGCCTAAACTTCAGCC
A	K699[b]	C1-N-1840	AGGAGGATAAACAGTTCA(C/T)CC
B	Ron II[a]	C1-J-1751d	GGAGCTCCAGATATAGCATTCCC
B	K525[a]	C1-N-2329	ACTGTAAATATATGATGAGCTCA
C	Jerry[a]	C1-J-2183a	CAACATTTATTTTGATTTTTTGG
C	Pthom1[c]	C1-N-2934	GTTTTCATTCAATCGATG
D	Pthom2[c]	C1-J-2761	ATACCACGTCGATACTCGGA
D	Barbara[a]	C1-N-3661	CCACAAATTTCTGAACATTGACCA

[a] Simon *et al.* 1994
[b] Sperling *et al.* 1995
[c] this study

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Chapter 3. Determination of stage-specific mortality factors for a leafmining sawfly (*Profenusa thomsoni*) using a stage-structured population model and classification trees.

Introduction

Invasive alien species are one of the most significant threats facing native ecosystems (Colautti *et al.* 2006; Langor *et al.* 2008; Mattson *et al.* 1994; Pimentel *et al.* 2000). One such species is the ambermarked birch leafminer *Profenusa thomsoni* (Konow) (Hymenoptera: Tenthredinidae) a native of the Palearctic that was introduced to North America in the early 20th century (Benson 1959; Smith 1971). Since that time it has become one of the most geographically widespread and abundant species of non-native insect attacking trees in North America (Chapter 2; Digweed *et al.* submitted; Snyder *et al.* 2007).

Profenusa thomsoni causes significant aesthetic damage to birch (*Betula* L.) (Digweed *et al.* 1997; Drouin and Wong 1984; Lindquist 1955; Martin 1960). Repeated infestations may also contribute to reduced tree vigor or premature tree mortality (pers. obs.; Hoch *et al.* 2000). The large and sustained outbreaks necessary to cause such effects have occurred in urban and native forests across the Northern United States and Canada since the 1920s (Benson 1959; Digweed *et al.* submitted; Martin 1960; Snyder *et al.* 2007). Despite this, *P. thomsoni* has only been studied for brief periods in Ontario (ON) in the 1950s, Alberta (AB) in the 1970s and 1990s (Digweed 1998a; Digweed 2006; Digweed *et al.* 2003; Drouin and Wong 1984; Lindquist 1959; Martin 1960; McQueen 1996; Watson 1959) and in New York (Pezzolesi and Hagar 1994).The dearth of research on *P. thomsoni* has likely contributed to the perception that it is a minor pest. In comparison, *Fenusa pumila* Leach, another equally-common, alien, birch leafmining sawfly has been much more extensively studied (Cheng and LeRoux 1965, 1966, 1969, 1970; DeClerck and Shorthouse 1985; Digweed 1998a; Eichhorn and Pschorn-Walker 1973; Fiori 1987; Fiori and Dolan 1984; Friend 1931; Fuester *et al.* 1984; Guèvremont and Quednau 1977; Hoch *et al.* 2000;

Jones and Raske 1976; Langor *et al.* 2000; van Driesche *et al.* 1997). In fact, *P. thomsoni* been misidentified as *F. pumila* (Martin 1960; Snyder *et al.* 2007), perhaps contributing to the misconception that *P. thomsoni* is a less important species particularly in those areas where both occur, i.e., AB and ON (Drouin and Wong 1984).

Recently, *P. thomsoni* was introduced to Alaska (AK) and rapidly became the most significant defoliator of birch in the state (Snyder *et al.* 2007). This outbreak fostered a need for pest management. However, as there was no information about the AK population in particular, and little about the species in general, it was important to examine the mortality factors affecting *P. thomsoni* in AK prior to the implementation of any control effort (e.g., Chapter 4). Such analyses were needed to provide a baseline to evaluate the impact of future pest management (Simberloff and Stiling 1996).

I studied an outbreak population of *P. thomsoni* in Anchorage, AK between 2003 and 2005 to determine the factors influencing its larval and egg mortality, and development time. I surveyed causes of egg and larval mortality and then determined the association between intraspecific competition, host and abiotic factors and larval mortality and quantified their effects using classification trees (Brieman *et al.* 1984; De'ath and Fabricius 2000). Classification tree analysis is a method of analyzing large ecological datasets that can reveal patterns between qualitative and quantitative predictor variables and categorical response variables (De'ath and Fabricius 2000). Recent studies have used classification tree analysis in ecological site classification, species presence/absence prediction and habitat selection studies (e.g. De'ath and Fabricius 2000; Usio *et al.* 2006). However, the method was first used in epidemiological studies to classify factors predicting human mortality and survivorship following medical treatments (e.g. Brieman *et al.* 1984; Therneau and Atkinson 1997). Herein, classification trees are applied to the investigation of insect mortality for the first time. I performed a life system analysis of *P. thomsoni* in Anchorage AK by fitting a stage-frequency model (Manly 1990) to population data to determine egg and larval survivorship, development time and the population size of each larval instar. These population parameters were then compared across years and among crown positions in the host trees to explore changes through time and test if variation within host trees had significant effects on *P. thomsoni* survivorship, development or population size.

Methods

Life cycle of *P. thomsoni* in Alaska

Profenusa thomsoni emerges between mid June and August in AK, after leaves on the host birch trees have fully flushed (Chapter 1). Almost immediately the parthenogenic females begin depositing eggs singly under the upper epidermis of birch leaves (Benson 1950, 1959; Martin 1960; Schönrogge and Altenhofer 1992). Each female will lay an average of c. 20 eggs, distributing them over several leaves (pers. obs.; Chapter 1; Digweed 2006). Eggs hatch c. 10 -12 days after being deposited. The larval stage has six instars (hereafter denoted L1–L6), the first five of which feed on the palisade mesophyll layer of the host leaf. After molting to L6, the larva chews a hole in the top or bottom of the leaf, drops to the ground and enters the soil. There, the larvae constructs a pupal cell where it over winters as an eonymph (Martin 1960; Watson 1959, Chapter 1). Pupation occurs in the pupal cell c. 1-2 weeks before the leafminer emerges the following spring (pers. obs.). Some individuals exhibit extended diapause, emerging as adults after a second winter spent as an eonymph in the pupal cell (pers. obs.; S Digweed pers. comm.). There is one generation per year.

Sampling

Populations of *P. thomsoni* were sampled at each of eight sites within the city limits of Anchorage, AK in 2003 (Figure 3.1). Two of these populations (JV, RJ; Figure 3.1) were re-sampled in 2004 and 2005. A single tree of either *Betula papyrifera* Marsh. or *B neoalaskana* Sarg. (5–10 m tall) was sampled at each site each year. A new tree was selected at those sites that were re-sampled in 2004 and 2005. Trees were selected randomly at each site but were chosen such that the upper crown could be reached with the pole pruners. All trees were situated in small stands (5–50 trees) of birch interspersed with small numbers of white spruce (*Picea glauca* [Moench] Voss), Sitka spruce (*Picea sitchensis* [Bong.] Carr.), balsam poplar (*Populus balsimifera* L.) or trembling aspen (*Populus tremuloides* Michx.). All sites had an under story of grass and small herbaceous plants, typical of urban parkland habitats in Anchorage (pers. obs.). Two sites were located adjacent to pedestrian pathways of either asphalt (BS, Figure 3.1) or gravel (OV, Figure 3.1) All the sampled trees were located at the edge of stands except the trees at sites LL and WL (Figure 3.1) which were surrounded by grass.

In all years, trees were sampled 13 to 17 times (i.e. every 3 to 4 days) between June and August. Frequent samples were taken to permit the construction of stage-frequency curves for use in subsequent analysis (see below). Each time a sample was taken a randomly selected branch segment containing 20+ leaves was clipped from each of the lower, middle and upper crown of each tree. This gave a total of three samples per site per sampling time. Branch segments were selected randomly with respect to cardinal orientation and clipped using a pole pruner but care was taken to avoid repeatedly sampling the same branch. At the lab all the leaves were removed from a branch segment, mixed and 15 leaves randomly drawn and examined in detail. Samples from each site and crown height were examined separately. For each leaf, I recorded the developmental stage (egg, L1-L6) and state (live or dead) of each individual of *P. thomsoni*. Larval instars were determined on the basis of size, relative head capsule width and the number of cast larval skins present in the mine. I also noted the cause of mortality for dead individuals. For eggs four categories of mortality were used; 'mechanically damaged' for those eggs that appeared crushed or deflated but did not show any discoloration or disruption to the upper epidermis of the leaf enclosing the egg; 'parasitized' for eggs that contained Trichogrammatidae (Hymenoptera: Chalcidoidea) which could be distinguished by the leafminer egg turning black or by the characteristic emergence hole made when the adult parasitoid ecloses; 'predated' for eggs that showed piercing or disruption of the upper leaf epidermis surrounding the egg; and 'unknown' for eggs that deflated and turned brown or for eggs that did not appear to die from the other causes listed. Similar categories were adopted for larvae; 'parasitized' for larva harbouring idiobiont ectoparastoids (no idiobiont endoparasitoids were found); 'predated' for those larvae showing feeding damage, e.g., ripped cuticle or piercing damage; an 'unknown' category was used to qualify the majority of larvae that did not fit into the other two categories. Finally, I visually estimated leafminer damage (% of leaf mined in 10% increments) and measured the length and width of each leaf, from which the leaf area was determined (e.g., Digweed 1998b; Sugiura *et al.* 2007; see footnote to Table 3.1).

Degree-Day Calculations

Developmental time was expressed in terms of accumulated degree-days above 5°C (DD_{05}) for all analyses. Digweed (1995) showed that this threshold was appropriate for *P. thomsoni* populations in AB. DD_{05} values were determined from the daily maximum and minimum temperatures using the single-sine method (Allen 1976) as implemented in the DEGDAY software (Snyder 2005). Temperature data for Anchorage were obtained from the United States of America's National Climatic Data Centre (National Climatic Data Centre 2006).

Life System Analysis

I used the total number of live eggs and larvae collected from each population and crown position on each sampling day (hereafter called "stage-frequencies") to perform a life system analysis of the *P. thomsoni* population in Anchorage by fitting the Kiritani-Nakasuji-Manly (KNM) model to field data. (Manly 1976; Manly 1990). The KNM model used the area under the stage-frequency curve to estimate the following three population parameters for each developmental stage (Southwood 1978): 1) *survivorship* - the proportion of individuals that survive a developmental stage; 2) *developmental time* - the length of time individuals spend in a developmental stage; and 3) the *number entering* - the total number of individuals that start each developmental stage (adapted from Manly 1976; Manly 1990). In the terminology of the model 'developmental stage' refers to the egg stage and six larval instars of *P. thomsoni* (after Manly 1990). More information on the development of this class of model and their application to the dynamics of stage-structured organisms can be found in Southwood (1978) and Manly (1990).

Two methods were used to assess the fit of the KNM model to the field data before the population parameters were used in subsequent analyses. The first method determined if the estimated parameters represented the larger population. This was done by simulated sampling of other populations equal in size to that from which the population parameters were calculated ('Method 4' Manly 1990). The second method evaluated the goodness-of-fit of stage-frequency data predicted by the KNM model to the stage-frequency data collected in the field ('Method 3' Manly 1990).

For the first method, 100 stage-frequency distributions were created and analyzed using the KNM model. Means were then calculated from the distributions of the resulting simulated population parameters. The KNM model was deemed a good fit if the population parameters derived from the observed stage-frequencies fell within 2 standard errors (2 SE) of the mean derived from the simulated data (Manly 1990).

The second method of model assessment used the calculated population parameters to predict stage frequency distributions. Starting with a population of eggs equal in size to that observed in the field, the population parameters were applied deterministically to obtain stage-frequency distributions. If the KNM model was appropriate to model the observed data, then parameters derived from it should have predicted stage-frequencies similar to those observed in the field. Differences between the observed and predicted stage-frequencies were assessed graphically by visual examination of the observed and predicted stage-frequency plots, and statistically using the root mean square error (RMS) to estimate the dissimilarity between the two distributions. This procedure was then repeated for each of the 100 simulated populations created using the first method to generate a range of RMS values. The RMS value for the observed data was then compared to the range of RMS values. If the RMS value for the observed data fell within the range of simulated RMS values the KNM model was deemed a good fit to the data (Manly 1990).

All model parameters and simulation analyses were computed using the P1F software (Manly 1994). However, no estimates of L6 survivorship or stage durations could be calculated. The KNM model uses sampling data from subsequent stages to calculate these measures, therefore, since the stage after L6 was not sampled, survivorship and stage duration could not be calculated for L6 larvae. In the results, all estimates of the number entering each stage are expressed relative to the size of the original sample, i.e., per 15 leaves.

Classification tree analysis of mortality factors

Introduction to method

Preliminary analysis of larval mortality indicated most individuals were killed by unknown causes. Therefore it was not possible to partition the effect of different causes on stage-specific mortality. To address this problem I used classification trees as a method to explore factors associated with individual larval mortality using (Brieman *et al.* 1984; De'ath and Fabricius 2000). Classification tree analysis attempts to explain the variation in a dataset with a categorical response variable by splitting the data into homogenous groups based on the values of predictor variables (De'ath and Fabricius 2000). To do this, the classification tree method uses an algorithm that can select predictor variables that are able to best split a given dataset into like-groups of response variables that are as homogenous as possible (De'ath and Fabricius 2000; Therneau and Atkinson 1997). The method is particularly suited for exploratory analysis because, unlike regression, it is insensitive to correlations and co-variation between predictor variables and can accommodate both categorical and continuous predictor variables (Brieman *et al.* 1984).

The result of a classification tree analysis is presented pictorially as a bifurcating tree. The values of the predictor variables are placed at the internal nodes and the groups of like-individuals are placed at the terminal nodes (e.g., Figure 3.2). The individuals in the groups at the terminal nodes share the value(s) of the predictor variable(s) at the internal node(s) above them. Each terminal node also indicates the dominant state (e.g., live or dead) of response variable of the individuals resolved at the node, the proportion of individuals in that state, and the number individuals resolved at the node. The number of terminal nodes on a classification tree (its ‘size’) and which predictor variables are retained are determined from the relative cross-validated error of the tree using the “1-SE” rule (Brieman *et al.* 1984). The relative cross-validated error is a measure of the tree’s ability to predict new data and the 1-SE rule is a ‘cut-off’ beyond which adding additional information (i.e. predictor variables) does not improve the quality of the tree’s predictions (Brieman *et al.* 1984; De'ath and Fabricius 2000; Therneau and Atkinson 1997). Once a tree’s size is determined, its success at partitioning data into homogenous groups can be evaluated using either misclassification rates (proportion of individuals placed in the wrong group) (Brieman *et al.* 1984; De'ath and Fabricius 2000) or the Kappa (K) statistic, an alternate evaluation of fit that describes the proportion of individuals correctly assigned by the classification tree that also accounts for the probability that some data can be correctly assigned by chance (Cohen 1960; Usio *et al.* 2006).

Mortality factor analysis

The dataset used for the classification tree analysis was comprised of the individual larvae sampled over the three years of the study. The response variable was the state (live or dead) of each larva and the predictor variables were based on competition (the total number of *P. thomsoni* in the same leaf and the total number of larvae in the same instar); host (leaf area, damage and crown position) and abiotic factors (year and DD_{05}) measured during the study (Table 3.1).

Classification trees were produced for each larval instar. For the purposes of this analysis, sampling times in terms of DD_{05} were expressed relative to the time larvae were first observed in a given year and grouped into increment classes (Table 3.1). Increment classes were used to group samples taken at the same approximate development time among the three years of the study. Also, because sampling times varied among years, grouping by similar DD_{05} reduced the likelihood that the classification tree algorithm would split the data based only on differences between sampling times.

The final size of each classification tree and the predictor variables that were retained was determined from the relative cross-validated error using the 1-SE rule after first constructing and comparing 25–50 classification trees to ensure that the selected tree size was not atypical (De'ath and Fabricius 2000). After the final tree size was determined, two misclassification rates were calculated for each classification tree. The 'null rate' was equal to the proportion of the least abundant class of response variable in the total data set (the 'go with the majority' rule, in this case the ratio of dead larvae to total larvae); the 'model rate' that was the error in the actual tree (De'ath and Fabricius 2000). K statistic values were also determined for each tree. Those values between 0.0–0.4 were considered to indicate poor assignment of data to the correct terminal node; 0.4–0.75 good assignment; and 0.75-1.0 excellent assignment (Usio *et al.* 2006). Trees were constructed in R (R Development Core Team 2007) using the rpart package (Therneau *et al.* 2007). Misclassification rates and K values were calculated by hand.

Two classification trees were constructed for each larval instar. At first classification trees were fit to the full set of all sampled larvae. However, these classification trees were a poor fit because the vast majority of larvae in that set were classed as 'alive', thereby masking the effect of dead larvae. Therefore, to better visualize patterns associated with mortality, a second analysis was performed using the subset of larvae from leaves containing at least one dead individual of *P. thomsoni*. This was expected to increase any 'signal' of mortality in the dataset by limiting the analyses to dead larvae and live larvae that experienced the same competition, host and abiotic conditions. I will refer to these classification trees as the 'full set' and subset' trees, respectively.

Statistics

Effect of year and crown position on population parameters

The effect of crown position and year on leafminer survivorship, developmental time and the number entering each stage was tested using the population parameters derived from the KNM model. The eight sampled populations were used as replicates; however only two populations were sampled across all three years. Therefore, each analysis was performed twice; one time with the full set of data and a second time with only those populations sampled in all years. All analyses were performed in SAS using PROC MIXED (SAS Institute Inc. 2000-2004) with year and height modeled as fixed effects and site as a random effect. When a significant effect of year was observed orthogonal contrasts were performed.

Results

Larval Sampling

Totals of 37,554 eggs; 4,835 L1; 2,806 L2; 2,500 L3; 2,721 L4; 2,117 L5 and 1,212 L6 were collected from 7,997 leaves over the three years of this study. The average area of sampled leaves was 12.4 ± 0.04 cm^2. Two proxy measures were used to enumerate L6 because few specimens were found: Presence of an L6 larva was recorded if; a mine was large enough to have produced a L6 and contained no other larvae, and had no evidence of predation damage; or if a mine contained a L5 skin with no accompanying L6.

Egg mortality was attributed to one of four causes: mechanical damage (e.g., crushed) (mean percent of total egg mortality ± 1 SE: 64.2% ± 5.5%, n=3 years); parasitism (7.4% ± 3.3%, n = 3); predation (1.7% ± 0.8%, n = 3) or unknown (26.5% ± 2.4%, n = 3). There cause of most larval mortality was unknown (mean percent of total larval mortality ± 1 SE: 96.4% ± 1.4%, n = 3 years) but a small proportion of larvae collected were depredated (0.65% ± 0.3%, n = 3) or parasitized (2.9% ± 1.5%, n = 3). No larval parasitoids were reared.

Classification tree analysis of mortality factors

Classification trees that analysed the subset of mortality records were a better fit to the data than classification trees based on the full set of larvae. Model misclassification rates for the subset classification trees were 8–12% better than the null rates compared to 1-6% better for the full set classification trees (Table 3.2). K values for the subset classification trees all fell within the 'good' range whereas values for the complete-set trees fell in the 'poor' range (Table 3.2).

Each of the subset classification trees had at least three terminal nodes and all analyses selected '% damage' or one of the competition variables (total stage, total leafminers) at the first internal node (i.e., splitting the greatest number of larvae, Figures 3.2–3.6). The L1 classification tree was the smallest of those produced, retaining two of the seven explanatory variables (Figure 3.2). Dead L1 larvae tended to occur where there were < 4.5 L1s per leaf or after 425 DD_{05} had elapsed.

The classification tree for L2 larvae retained six of the predictor variables (Figure 3.3) and indicated that dead L2s tended to be found in leaves with were < 2.5 L2 per leaf and > 75% damage. Dead L2 larvae in leaves with < 75% damage were split based on the year of collection; in 2004 dead L2s tended to occur in leaves > 15.2 cm^2; in 2003 and 2005, dead L2s tended to occur in leaves: with 25–75% damage; < 10.5 total leafminers; < 14.0 cm^2; and before 425 DD_{05} had elapsed (Figure 3.3, left side of tree). Dead L2 larvae associated with leaves having > 2.5 L2s (Figure 3.3, right side of tree) tended to be found in leaves also with > 75% damage and that were > 12.2 cm^2.

The classification tree for L3 larvae retained five predictor variables (Figure 3.4) and indicated that dead L3 larvae tended to occur in leaves with > 95% damage that were < 12.5 cm^2. However, some dead L3s were associated with leaves > 12.5 cm^2 but only those sampled from the middle and upper portion of the crown (Figure 3.4, left side of tree). Dead L3 larvae in leaves that were 65–95% damaged tended to occur in leaves < 12.7 cm^2 that harbored < 3.5 leafminers per leaf (Figure 3.4, centre of tree). However, while most L3s associated with leaves with < 65% damage were alive, all those collected after 625 DD_{05} were dead (Figure 3.4, right side of tree).

The classification tree for L4 larvae retained four predictor variables (Figure 3.5) and indicated that dead L4 tended to occur in leaves where damage was > 95%, except in 2003 when dead larvae tended to be found after 425 DD_{05} in leaves < 9.4 cm^2 (Figure 3.5, left side of tree). Dead L4 larvae in leaves with < 95% damage tended to occur where there were < 3.5 leafminers per leaf or after 625 DD_{05} (Figure 3.5, right side of tree).

The classification tree for L5 larvae was the only analysis to retain all seven predictor variables and was also the largest and most complex of all trees (Figure 3.6). Dead L5 larvae tended to occur where there were < 3.5 leafminers per leaf and > 95% damage (Figure 3.6, left side of tree). Dead L5s in leaves with < 3.5 leafminers but < 95% damage tended to occur after 425 DD_{05}. However, similar to the trees constructed for L2s and L4s (Figures 3.2 and 3.4), L5s sampled in 2003 were split into a separate set of terminal nodes. Dead larvae resolved in those nodes were associated with the lower crown and with leaves < 13.6 cm^2 (Figure 3.6, right side of left branch of tree). A similar pattern was seen in those terminal nodes descending to the right of the first internal node (i.e. those with > 3.5 leafminers per leaf; Figure 3.6, right branch of tree). In that group, dead L5 in leaves with > 3.5 leafminers per leaf tended to occur in leaves with > 95% damage. Again, except in 2003 when dead L5s were associated with leaves < 7.6 cm^2 or leaves > 7.6 cm^2 containing > 5.5 L5 (Figure 3.6, left side of right branch of tree).

No classification tree analysis was possible for L6 larvae.

Life system analysis

The temporal distribution of egg and larval instars collected over the three years of the study that were used to derive the KNM model estimates are presented in Appendix 1. Stage-specific survivorship, developmental time and the number entering each stage were determined for eggs and L1–L6 larvae for all sites and crown positions except for the RJ site in 2003 (lower crown: no parameters for L4–L6 larvae; middle crown: no estimates for L5s and L6s; see Appendix 2). Both methods of model assessment suggested that the KNM model was a reasonable fit to the observed data. Of 211 individual stage-specific survivorships calculated only 10% (21) fell outside the simulated distribution of population parameters (i.e., were more than 2 SE; see Appendix 2). Likewise, only 11% (23) of 209 stage duration estimates fell outside the simulated distribution (Appendix 2). Estimates of number entering each stage were less well predicted as 21%

(52) of 247 fell more than 2 SE from the simulated mean (Appendix 2). No RMS scores were outside the simulated range (Appendix 2) but the graphical comparisons of the expected and observed stage-frequencies suggested the model was a better fit for early developmental stages (eggs–L2) than for later stages (L3–L5; see Appendix 3).

Effect of year and crown position on population parameters

I observed year-to-year variation for all population parameters (Table 3.3). However, there was no significant effect of crown position on any population parameter for any developmental stage nor did the effect of crown position vary among years (i.e., no significant year by crown position interaction; see Table 3.3). Survivorship followed the same trend in all three years: Eggs, L4s and L5s had the lowest survival (Figure 3.7A, E, F) whereas survivorship of L1s, L2s and L3s was always higher (Figure 3.7B, C, D). Survivorship was highest in 2004 for most stages but there was no difference between 2004 and 2005 for either eggs or L1s (Figure 3.7A, B). Development time varied among stages but not among years for most stages (Figure 3.8). The exception was L1 larvae which had longer development times in 2003 than in 2004 (Figure 3.8). There was little year-to-year variation in the number entering each stage (Figure 3.9). However, more L6s were observed in 2004 than in 2003 and 2005 (Figure 3.9G) and more eggs were observed in 2003 than in 2004 and 2005 (Figure 3.9A).

Almost identical results to those described above were observed in the partial set comprised of just those sites sampled in all three years (Table 3.3, Figures 3.7H-M, 3.8H-M, 3.9H-M). However, unlike the full dataset, L4 and L5 survivorship and L4 development time did not vary among years (Table 3.3).

Discussion

Survivorship of *P. thomsoni* eggs and larvae in Anchorage is high and there is little population regulation by natural enemies or other extrinsic factors. The mortality that does occur appears to be related to a combination of competition and within leaf resource depletion, with abiotic factors exerting less influence. Moreover, the combination of factors contributing to mortality seems to be unique to each stage. It also appears that application of classification tree analysis to define these combinations is a useful technique for study of insect mortality.

Life-system analysis

Overall survivorship of *P. thomsoni* larvae in Anchorage was high, but survivorship varied among individual stages. Egg survivorship may have been low because the egg development period exposed this stage to potential mortality agents for a longer period compared to later stages. The duration of the L4 and L5 stages was longer than that of L1–L3 stages but their mortality appears to be best predicted by a combination of resource limitation and competition. Furthermore, the pattern of survivorship was similar among the stages over the three years of the study, even in 2004 when survivorship of all stages was highest. This consistency among years suggests that, in the absence of a new mortality agent, the population dynamics of *P. thomsoni* in AK are unlikely to change in the near-term.

The KNM model derived an estimate that indicated c. 1–2 leafminers are produced per-leaf per-year. This estimate supports earlier observations that there is over-utilization of the host plant material by *P. thomsoni* during outbreaks because most leaves harbour more than two larvae (Digweed 2006; Martin 1960). Moreover, the model results are supported by field observations that most fully defoliated leaves only produced one or two L6 larvae. The modelled and observed L6 production per-leaf in AK also coincide with the predicted L6 production per-leaf based on the estimation that each *P. thomsoni* larva requires 5.3 cm^2 of leaf tissue to complete development in ON (Martin 1960). Taken together, these three pieces of evidence suggest that c. 2 leafminers per-leaf per-year is the maximum per-leaf production of L6. However, in AB *P. thomsoni* larvae require 2.8 cm^2 of leaf tissue to complete development (Digweed *et al.*

1997). Such regional variation suggests additional examination of *P. thomsoni* production in AK would be interesting.

If an estimate of c. 2 leafminers per-leaf per-year is accurate for birch trees in AK then managers may be able to use this value to estimate when leafminer population levels are approaching a maximum. By combining estimates of per-leaf leafminer production with measures of % defoliation and estimates of per-tree leaf production it should also be possible to generate reasonable estimates of the number of leafminers produced per-tree per-year. Moreover, by combining those estimates with measures of percent parasitism (e.g., Chapter 4) it would be possible to estimate the local population size of these natural enemies, especially in the context of monitoring the success of introductions for biological control.

Mortality factors affecting birch leafminers elsewhere do not appear to have an influence on the dynamics of *P. thomsoni* in AK. Most apparent was that parasitoids and leaf abscission were not significant factors as they are for birch leafminers in Europe and for most leafmining insects in general (Auerbach and Simberloff 1989; Connor and Taverner 1997; Eichhorn and Pschorn-Walker 1973; Hespenheide 1991; Pschorn-Walker and Altenhofer 1989; Schönrogge and Altenhofer 1992). Birches in AK do not abscise mined leaves although fully mined leaves do fall off trees earlier (pers. obs.), and, with the exception of a generalist egg parasitoid, there appear to be no native natural enemies of *P. thomsoni* in AK. Moreover, while it is possible that ants (Hymenoptera: Formicidae) or birds may become significant natural enemies of L1-L5 *P. thomsoni* as they are elsewhere against other birch leafminers (Cheng and LeRoux 1966, 1969, 1970; Digweed 1998a; McQueen 1996; Pezzolesi and Hagar 1994) this has not yet occurred in AK.

Within-tree variation in patterns of mortality due to intraspecific competition also differed in AK from those seen elsewhere. Martin (1960) noted less mortality due to overcrowding in the middle and upper crowns of birch in ON which he attributed to females preference to oviposit in the lower crown. In AK however, I observed no such preference and consequently survivorship did not differ among crown heights. However, I did observe that females began ovipositing in the middle and upper crown later than in the lower crown, but this does not appear to have had an effect on survivorship.

There is little information from any jurisdiction about mortality of L6 *P. thomsoni* larvae after they depart the leaf and no information about the mortality of the eonymph, pupal and adult stages. Likewise, the mortality of L6 larvae after they departed the leaf was not measured in this study. However it is probable that some were predated on before they constructed pupal cells, particularly by ants, as was seen for *F. pumila* in Quebec and *P. thomsoni* in AB and ON (pers. obs. Cheng and LeRoux 1969, 1970; Martin 1960; McQueen 1996). Observations in AK suggested there was little effect of natural enemies on pupae and eonymph survivorship (Appendix 4). However, vertebrate predators have been shown to significantly impact other leafminers that overwinter in the soil (Buckner 1959). There is no accurate way of measuring adult mortality during dispersal and oviposition and thus it will be difficult to establish the role of adult mortality of *P. thomsoni* population dynamics.

In addition to differing from other leafminer species, *P. thomsoni* in AK also differed from other North American populations of birch leafmining sawflies. Some of these differences may be due to the short time the leafminer has been in AK. Previous studies were conducted on populations that had been established for at least 20 years (Cheng and LeRoux 1965, 1966, 1969, 1970; Digweed 1998a; Digweed 2006; Drouin and Wong 1984; Martin 1960). In comparison, by 2003 *P. thomsoni* had been present in AK for 15 years and in Anchorage (site of my study) for no more than 10 years (Snyder *et al.* 2007). Thus native natural enemies may have had insufficient time to adopt *P. thomsoni* as a host, as seems to have occurred in AB (Digweed *et al.* 2003). Furthermore, native birches in AK may not have developed resistance to *P. thomsoni*, even though they may express some resistance to the native birch feeder, spear-marked black moth, *Rheumaptera hastata* L. (Lepidoptera: Geometridae) (Bryant *et al.* 1993). Lastly, inter- and intraspecific competition appear to play a minor role in regulating *P. thomsoni* populations. However, significant competition to *P. thomsoni* could come from the birch leaf roller, *Epinotia solandriana* L. (Lepidoptera: Tortricidae), a species that occasionally has large outbreaks in AK (Wittwer 2003), or the birch skeletonizer, *Bucculatrix canadensisella* Chambers (Lepidoptera: Bucculatricidae) which occurs in AK and has been shown to impact *P. thomsoni* populations in AB (Digweed 1998b). Other alien birch leafmining sawflies, specifically *F. pumila* and *Heterarthrus nemoratus* Fallén (Hymenoptera: Tenthredinidae) have been found in AK (Snyder *et al.* 2007), but these species are too rare at present to provide much competition for *P. thomsoni*. However, in time they could become direct or indirect competitors of *P. thomsoni* as was evident in AB (Digweed 1998a; Drouin and Wong 1984).

Influence of mortality factors

Intraspecific competition and within leaf resource limitation were the most important variables predicting *P. thomsoni* mortality in AK. Similar results were reported for populations in AB (Digweed 2006). In both studies, more eggs were oviposited in leaves than could possibly complete development (i.e., a maximum of 1-2 larvae per leaf). This behaviour is common among leafminer species and is hypothesised to be a response to parasitoid induced mortality. Parasitoids regulate many leafminer populations below the level where intraspecific competition would occur (Faeth and Simberloff 1981). This may reduce selective pressure for the evolution of a means to avoid conspecifics during oviposition. Thus any intraspecific competition that does occur is expected to be exploitative (Hespenheide 1991). The over-utilization of birch leaves for oviposition by *P. thomsoni* may therefore reflect adaptation to selective pressures in the Palearctic (its native range) where it is attacked by at least one parasitoid (Fisher 1997; Kenis and Carl 1995; Kenis *et al.* 1995; Schönrogge and Altenhofer 1992).

An alternate explanation for over-utilization of birch leaves for oviposition by *P. thomsoni* is that group feeding may be advantageous for young larvae. This hypothesis is suggested by my classification tree analysis showing live L2 and L3 were associated with higher numbers of leafminers. This pattern suggests larger groups may have an advantage over smaller groups. If there is an advantage to group feeding it may be that the effect of plant defences on individuals is minimized. In the Palearctic, birches produce phenolics and other compounds that deter herbivory by externally feeding sawflies (Haukioja 2003). Birches in AK produce similar compounds in response to herbivory by Lepidoptera (Bryant *et al.* 1993). Group feeding by young *P. thomsoni* larvae could minimize the effect of these compounds by ensuring that leaves are consumed before the tree can respond or by disrupting local delivery of defensive compounds through effects on the leaf's vascular system. If so, this could result in increased survivorship of larvae feeding in groups compared to individual larvae feeding alone. This hypothesis, of an adaptive advantage for group feeding in *P. thomson*i, has not been tested in AK but is supported by the observation that females favour leaves containing eggs, and that the weight of surviving larvae decreases only slightly with increasing density in AB (Digweed 2006). Furthermore if group feeding evolved after *P. thomsoni* was introduced to North America it may explain why outbreaks and significant damage are more common in North America than in the Palearctic. (Schönrogge and Altenhofer 1992).

The classification tree analysis also suggested a relationship between leaf area and mortality. In all but one classification tree the splitting criteria for leaf area fell between 7.9 cm^2 and 15.2 cm^2, with dead larvae associated with smaller leaves. Leaf surface flavonoids are more concentrated in small leaves of the Palearctic species *B. pendula* and *B. pubescens* (Valkama *et al.* 2004). Concentration of flavinoids or similar compounds in small leaves of Nearctic birches could explain why dead larvae were associated with small leaves in this study. Alternately, competition could be more intense in smaller leaves as females do not react to leaf size when selecting oviposition sites (Digweed 2006). In either case the negative relationship between leaf area and mortality suggests there is a critical size for leaves below which mortality is more likely to occur. This hypothesis merits testing in North American urban birches as it may suggest a source of potential resistance to *P. thomsoni* damage or oviposition (sensu Fiori 1987; Fiori and Dolan 1984; Hoch *et al.* 2000).

Use of classification trees in the analysis of insect mortality

This study has demonstrated that classification trees can be used to effectively analyze insect mortality data. Moreover they may also provide insight about specific biological features such as the possible advantage of group feeding in *P. thomsoni* as discussed above. In addition this analytical approach may reveal relationships obscured by the variability inherent to large data sets, e.g., year-to-year variation in mortality patterns for L2, L4 and L5. Rigorous manipulative experimentation will be more effective at demonstrating specific relationships between species and their environment but the relative merit of hypotheses can be assessed using classification trees. Thus, classification trees may be useful in the study of organisms about which little is known, e.g.,) alien or newly invasive species.

The ultimate utility of classification trees depends on the selection of good predictor variables. Herein, I selected predictor variables that are known to influence mortality of other leafmining insects and insects that feed on birch (e.g., Connor and Taverner 1997; Hartley 1988; Hartley and Lawton 1987; Haukioja 2003; Hespenheide 1991; Tuomi *et al.* 1981). This approach should prevent testing of variables that may be unimportant for providing biological insight. Classification tree analysis can be used as a tool to winnow out variables that are not biologically important for the species of interest (e.g., DD_{05}, year, crown height), from those that are (e.g., competition, leaf area). Furthermore, I offer the caveat that any relationship revealed in the classification tree should be interpreted with caution. Without subsequent testing, or independent support, relationships revealed in a classification tree are analogous to correlations. Thus, classification trees should be considered as hypotheses consistent with the data that should be given high priority for experiments.

Conclusion

This study is a snapshot of the recently established population of the alien invasive species *P. thomsoni*, in Anchorage, Alaska prior to the establishment of a biological control agent (Chapter 4). In general such studies of a newly invaded species are rare but are necessary to understand the biology of invasive species. Life-table data from this work will support subsequent evaluations to determine if releases of potential biological control agents, such as *Lathrolestes luteolator*[1] Gravenhorst (Hymenoptera: Ichneumonidae), are successful in suppressing *P. thomsoni*. Moreover, The factors that currently influence the population of *P. thomsoni* in Anchorage differ from that observed in other populations of birch leafmining sawflies. I hypothesized that some of these differences are related to the short period this species has been resident in AK. It is likely that along with biological control agents other mortality factors will become important in suppressing *P. thomsoni* the longer it is resident in Alaska. To evaluate this hypothesis, it will be necessary to re-evaluate the dynamics of *P. thomsoni* populations in AK at regular intervals to quantify changes that may occur. For instance, birds and other natural enemies may adopt *P. thomsoni* as prey; *L. luteolator* or other parasitoids may become significant mortality factors; other leafminers may invade, increasing competition for limited resources; or exogenous features, such as climate change, could alter the linkage between birch and leafminer phenologies. Evolutionary and behavioural adaptations can be expected to occur in both invasive species and native species that could significantly alter population dynamics (e.g., Elkinton *et al.* 1996). This chapter highlights the importance of studying invasive species at various points along the invasion continuum.

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[1] See footnote to Chapter 1 on page 5.

In addition to summarizing the life-system parameters of the Anchorage population of *P. thomsoni* I have shown that the KNM model can be used to derive estimates of insect survivorship and development as well as changes in population size. This technique likely has utility for other invasive species, and in future monitoring efforts of *P. thomsoni* in Alaska. It is also probable that data could be collected at a lower frequency than was done here (perhaps weekly) without compromising interpretations and thus supporting work to generate data sets that can be compared in space and time. With a small amount of additional effort, it would also be possible, for example, to apply the classification tree analysis to monitor shifts in the influence of mortality factors that may follow biological control attempts.

Table 3.1 Variables used in classification tree construction

Variable name	Description	Values
Response		
status	Health of larva	Live, Dead
Competition		
total stage	Number of *P. thomsoni* larvae of the same instar in the same leaf	1 – 22 (L1) 1 – 17 (L2) 1 – 12 (L3) 1 – 11 (L4) 1 – 9 (L5)
total leafminers	Number of *P. thomsoni* larvae present in the same leaf	1 – 36
Host		
leaf area*	Area of the sampled leaf	1.0 – 33.2 cm^2
damage	Percentage of leaf tissue consumed by leafminers	0 – 100% in 10% increments
position	Position within the crown of the tree from which sample was taken	Lower, Middle, Upper
Abiotic		
year	Year sample was taken	2003, 2004, 2005
acc degree-day	Degree-days above a 5°C threshold accumulated by the larva. Calculated relative to the time larvae were first observed in a given year	0 – 650 DD_{05} in 50 DD_{05} increments

*Leaf area = 0.5582x + 61.402 where x is the ratio of leaf width to leaf length (CM, unpublished)

Table 3.2 Results of classification tree analysis of individual *Profenusa thomsoni* larval mortality for two related data sets. The full set (F) of larvae from all sampled leaves and the subset (S) of larvae from leaves containing at least one dead larva.

Stage	Set	sample size	Misclassification rates		K*	Tree size (no. terminal nodes)	Variables retained†
			null	model			
L1	F	4834	31	25	0.32	3	acc degree-day
	S	2392	36	28	0.38	3	total stage, acc degree-day
L2	F	2805	15	12	0.32	7	damage, acc degree-day, leaf area
	S	1345	32	20	0.47	11	total stage, damage, leaf area, year, total leafminers, acc degree-day
L3	F	2500	12	10	0.36	5	damage, acc degree-day
	S	1238	24	16	0.47	10	damage, acc degree-day, total leafminers, leaf area, position,
L4	F	2721	12	9	0.46	12	damage, acc degree-day, total stage, total leafminers, leaf area
	S	1052	30	19	0.47	7	damage, year, total leafminers, acc degree-day, leaf area
L5	F	2117	14	13	0.15	5	damage, leaf area, position
	S	1148	26	18	0.46	12	total leafminers, damage, acc degree-day, year, position, leaf area, total stage

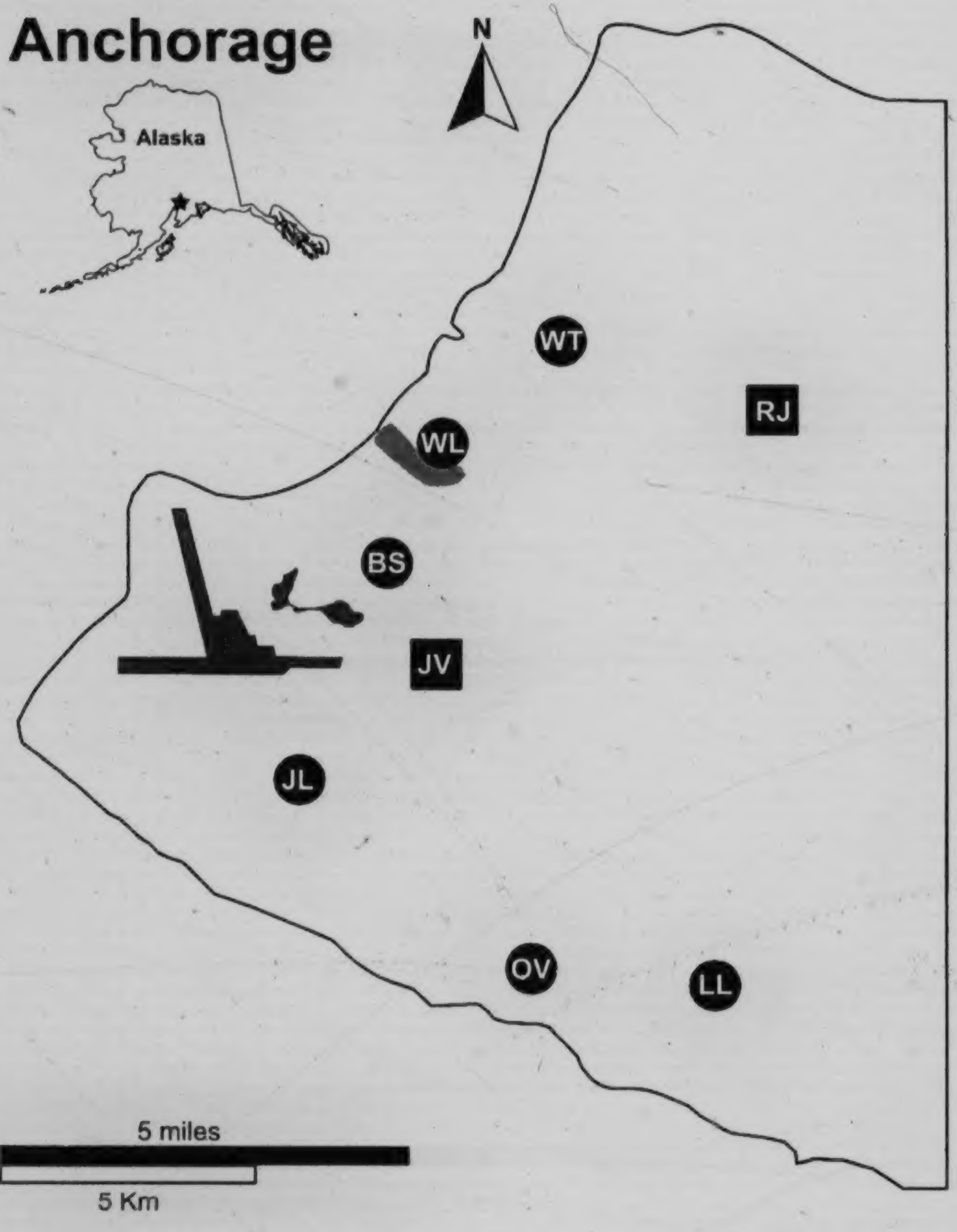
*Kappa; 0.0-0.4 poor assignment; 0.4-0.75 good assignment; 0.75-1.0 excellent assignment.

† ordered as they appeared in the classification tree

Table 3.3 Results of ANOVA for effect of year and crown position on stage specific survivorship, stage duration and number entering stage using population parameters from all sampled sites and only those sites sampled in all years. All values determined using PROC MIXED (SAS ver 9.0) with year and height modeled as fixed effects and site as a random effect. Bold text indicates significant p values. L1 = first instar, L2 = second instar, etc...

		stage-specific survival rate			stage duration			number entering stage		
	num df	den df	F	*p*	den df	F	*p*	den df	F	*p*
All Sample sites										
Egg										
year	2	20	16.23	**<0.001**	20	10.03	**0.001**	20	6.37	**0.007**
position	2	20	0.71	0.502	20	1.72	0.204	20	2.52	0.105
year*position	4	20	0.76	0.563	20	2.10	0.118	20	0.88	0.491
L1										
year	2	20	5.85	**0.010**	20	2.45	0.112	20	1.74	0.201
position	2	20	0.16	0.852	20	0.04	0.961	20	2.00	0.161
year*position	4	20	0.19	0.941	20	0.24	0.914	20	0.76	0.566
L2										
year	2	20	6.32	**0.008**	20	0.52	0.600	20	1.28	0.300
position	2	20	0.22	0.801	20	0.07	0.932	20	1.45	0.258
year*position	4	20	0.33	0.856	20	0.24	0.915	20	0.49	0.746
L3										
year	2	19	2.94	0.077	19	0.44	0.650	20	0.66	0.530
position	2	19	0.12	0.888	19	0.07	0.936	20	1.06	0.364
year*position	4	19	0.13	0.969	19	0.34	0.845	20	0.36	0.837
L4										
year	2	18	4.78	**0.022**	18	4.41	0.028	19	0.23	0.793
position	2	18	0.99	0.390	18	0.53	0.597	19	1.61	0.226
year*position	4	18	0.31	0.867	18	1.10	0.387	19	0.59	0.674
L5										
year	2	18	5.08	**0.018**	18	1.40	0.272	18	0.16	0.855
position	2	18	0.86	0.440	18	1.41	0.271	18	0.88	0.434
year*position	4	18	0.44	0.778	18	0.34	0.849	18	0.76	0.566
L6										
year	2							18	4.67	0.023
position	2							18	0.08	0.923
year*position	4							18	1.04	0.413
Sites sampled in all years										
Egg										
year	2	8	10.85	**0.005**	8	7.90	0.013	8	5.46	0.320
position	2	8	0.55	0.597	8	0.31	0.743	8	1.28	0.330
year*position	4	8	0.46	0.766	8	1.75	0.232	8	0.76	0.582
L1										
year	2	8	5.25	**0.035**	8	2.89	0.114	8	1.41	0.299
position	2	8	0.37	0.699	8	0.17	0.843	8	0.72	0.517
year*position	4	8	0.14	0.961	8	0.37	0.821	8	0.95	0.482
L2										
year	2	8	14.02	**0.006**	8	1.12	0.372	8	1.12	0.372
position	2	8	4.10	0.077	8	0.40	0.681	8	0.45	0.651
year*position	4	8	2.95	0.124	8	0.33	0.850	8	0.58	0.688
L3										
year	2	7	2.63	0.140	7	0.85	0.466	8	0.82	0.476
position	2	7	0.21	0.814	7	0.16	0.858	8	0.27	0.771
year*position	4	7	0.08	0.985	7	0.24	0.905	8	0.36	0.831
L4										
year	2	6	3.38	0.104	6	2.04	0.211	7	0.30	0.748
position	2	6	0.37	0.705	6	0.11	0.898	7	1.05	0.400
year*position	4	6	0.20	0.931	6	0.60	0.674	7	0.51	0.730
L5										
year	2	6	3.69	0.090	6	0.01	0.987	6	0.14	0.873
position	2	6	0.57	0.593	6	0.89	0.460	6	0.61	0.574
year*position	4	6	0.17	0.946	6	0.29	0.875	6	0.34	0.844
L6										
year	2							6	3.90	0.082
position	2							6	0.18	0.838
year*position	4							6	0.33	0.850

Figure 3.1 Location of study sites in Anchorage, Alaska. The outline map shows the Anchorage city limits. Anchorage is bordered on the north, west and south by Cook Inlet and on the east by the Chugach Mountains. Circles indicate sites sampled in 2003 only; squares indicate sites sampled in all years (2003-2005). Site names; BS: Balto Seppela Park, JL: Jewel Lake Park, JV: Javier de la Vega Park, LL: Loch Loman Lane, OV: Oceanview Bluff Park, RJ: Russian Jack Springs Golf Course, WL: Westchester Lagoon disc golf course, WT: Water Tower (on Harvard Road). Inset map shows location of Anchorage in Alaska.


Anchorage
Alaska
N
WT
RJ
WL
BS
JV
JL
OV
LL
5 miles
5 Km

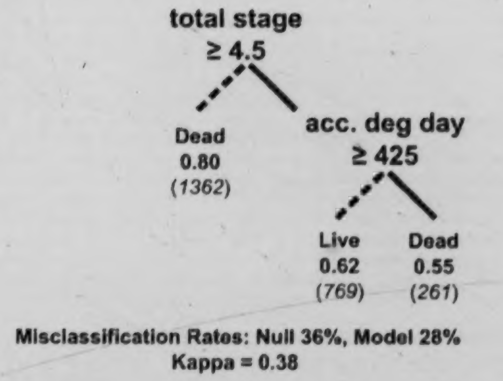


Figure 3.2 Classification tree depicting mortality factors influencing first instar *Profenusa thomsoni* larvae. Internal nodes are labeled with their splitting criteria, solid lines indicate descendant branches agree with the criteria at the node, dashed lines indicate the descendant branches disagree. Terminal nodes are labeled with the dominant state (live or dead) of the population of larvae resolved at the leaf, the proportion of larvae in that class (in bold), and the number of larvae in the group (in *italics*). Final tree size was selected based on the 1-SE rule. Misclassification rates indicate the proportion of larvae incorrectly assigned to the wrong state. The null misclassification rate was calculated based on a 'go with the majority' rule (see text); the model misclassification rate is the error rate of the depicted classification tree. Kappa is the proportion of larvae assigned to the correct state when the probability of a chance assignment has been removed. Kappa values of 0.0—0.4 indicate 'poor' assignment, 0.4—0.75 good assignment and 0.75—1.0 excellent assignment.

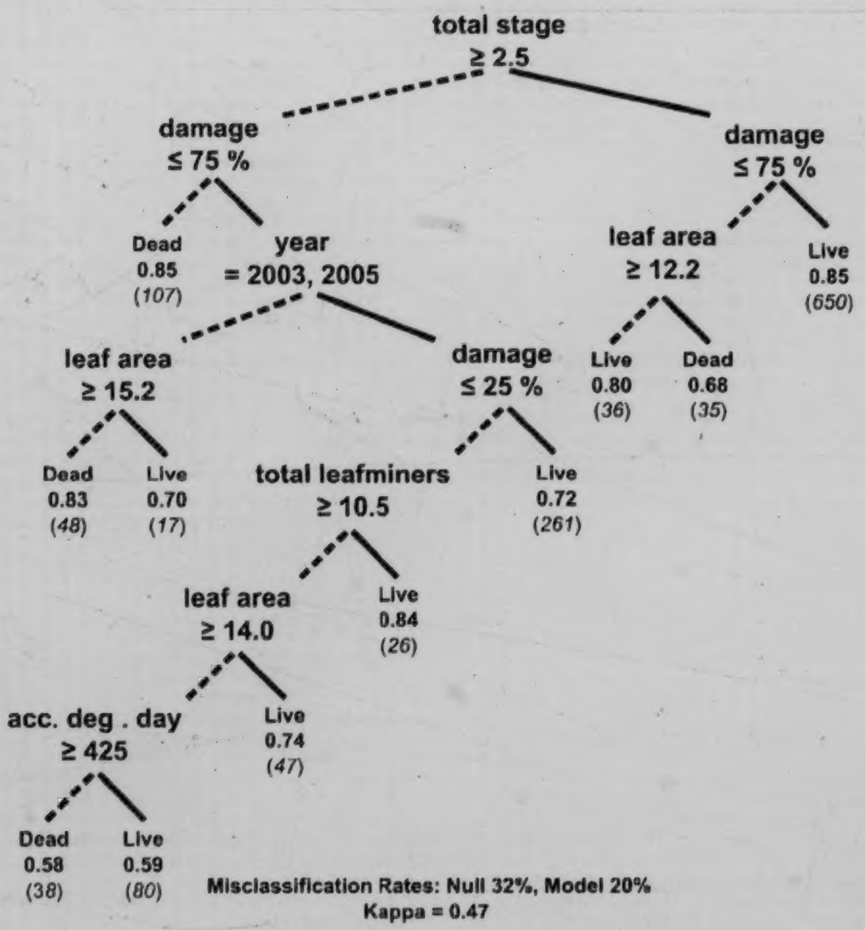

total stage
≥ 2.5
damage
≤ 75 %
damage
≤ 75 %
Dead
0.85
(107)
year
= 2003, 2005
leaf area
≥ 12.2
Live
0.85
(650)
leaf area
≥ 15.2
damage
≤ 25 %
Live
0.80
(36)
Dead
0.68
(35)
Dead
0.83
(48)
Live
0.70
(17)
total leafminers
≥ 10.5
Live
0.72
(261)
leaf area
≥ 14.0
Live
0.84
(26)
acc. deg . day
≥ 425
Live
0.74
(47)
Dead
0.58
(38)
Live
0.59
(80)
Misclassification Rates: Null 32%, Model 20%
Kappa = 0.47

Figure 3.3 Classification tree depicting mortality factors influencing second instar *Profenusa thomsoni* larvae. See caption for Figure 3.2 for more information.

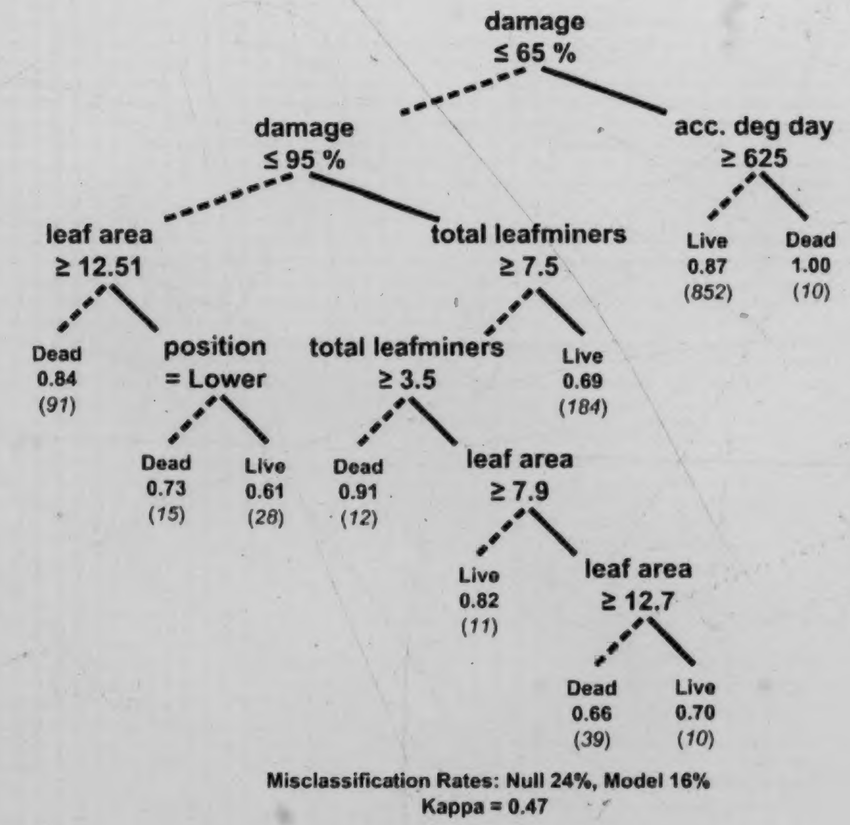

damage
≤ 65 %
damage
≤ 95 %
acc. deg day
≥ 625
leaf area
≥ 12.51
total leafminers
≥ 7.5
Live
0.87
(852)
Dead
1.00
(10)
Dead
0.84
(91)
position
= Lower
total leafminers
≥ 3.5
Live
0.69
(184)
Dead
0.73
(15)
Live
0.61
(28)
Dead
0.91
(12)
leaf area
≥ 7.9
Live
0.82
(11)
leaf area
≥ 12.7
Dead
0.66
(39)
Live
0.70
(10)
Misclassification Rates: Null 24%, Model 16%
Kappa = 0.47

Figure 3.4 Classification tree depicting mortality factors influencing third instar *Profenusa thomsoni* larvae. See caption for Figure 3.2 for more information.

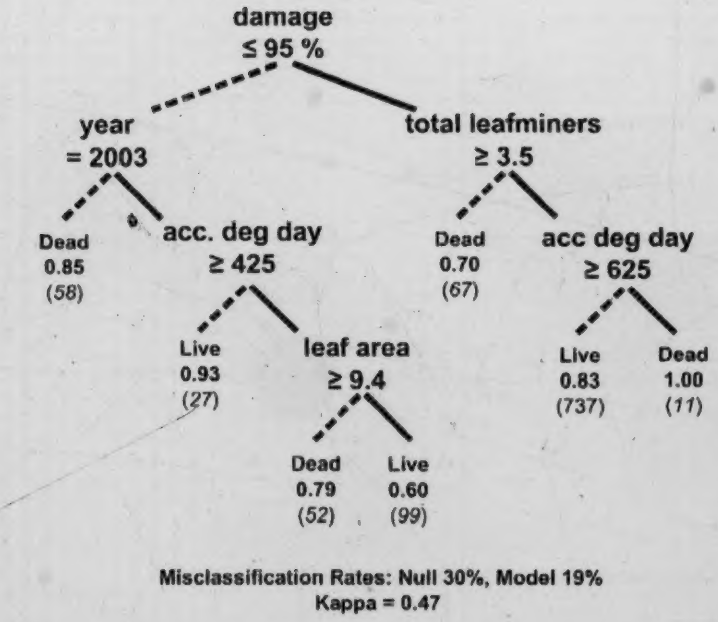
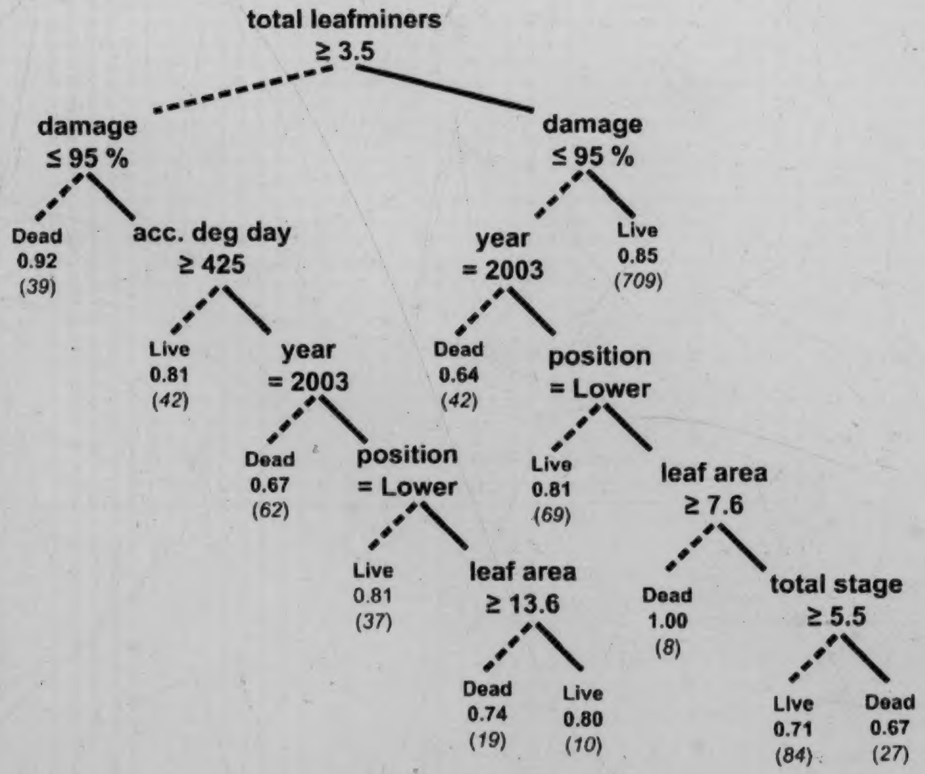

damage
≤ 95 %
year
= 2003
total leafminers
≥ 3.5
Dead
0.85
(58)
acc. deg day
≥ 425
Dead
0.70
(67)
acc deg day
≥ 625
Live
0.93
(27)
leaf area
≥ 9.4
Live
0.83
(737)
Dead
1.00
(11)
Dead
0.79
(52)
Live
0.60
(99)
Misclassification Rates: Null 30%, Model 19%
Kappa = 0.47

Figure 3.5 Classification tree depicting mortality factors influencing fourth instar *Profenusa thomsoni* larvae. See caption for Figure 3.2 for more information


total leafminers
≥ 3.5
damage
≤ 95 %
damage
≤ 95 %
Dead
0.92
(39)
acc. deg day
≥ 425
year
= 2003
Live
0.85
(709)
Live
0.81
(42)
year
= 2003
Dead
0.64
(42)
position
= Lower
Dead
0.67
(62)
position
= Lower
Live
0.81
(69)
leaf area
≥ 7.6
Live
0.81
(37)
leaf area
≥ 13.6
Dead
1.00
(8)
total stage
≥ 5.5
Dead
0.74
(19)
Live
0.80
(10)
Live
0.71
(84)
Dead
0.67
(27)

Misclassification Rates: Null: 26%, Model 18%
Kappa = 0.46

Figure 3.6 Classification tree depicting mortality factors influencing fifth instar *Profenusa thomsoni* larvae. See caption for Figure 3.2 for more information.

Figure 3.7 Stage specific survival rate of *Profenusa thomsoni* eggs and larvae in Anchorage, Alaska, 2003–2005. All values determined using the KNM model for stage frequency data. Panels A-F show least square means determined from the complete data set (eight sites were sampled in 2003, two sites were sampled in 2004 and 2005, least square means are shown to account for the unbalanced design); Panels H-M show means determined from just those sites sampled in all years. In all panels asterisks indicate a significant effect of year on the given parameter for a stage and lowercase letters indicate significantly different means. Contrasts performed using PROC MIXED (SAS Institute Inc. 2000-2004) with year and crown position as modelled as fixed effects and site as a random effect with year versus year orthogonal contrasts after a significant effect was observed. Note that panel G is missing to allow comparison between this figure and Figure 3.9.

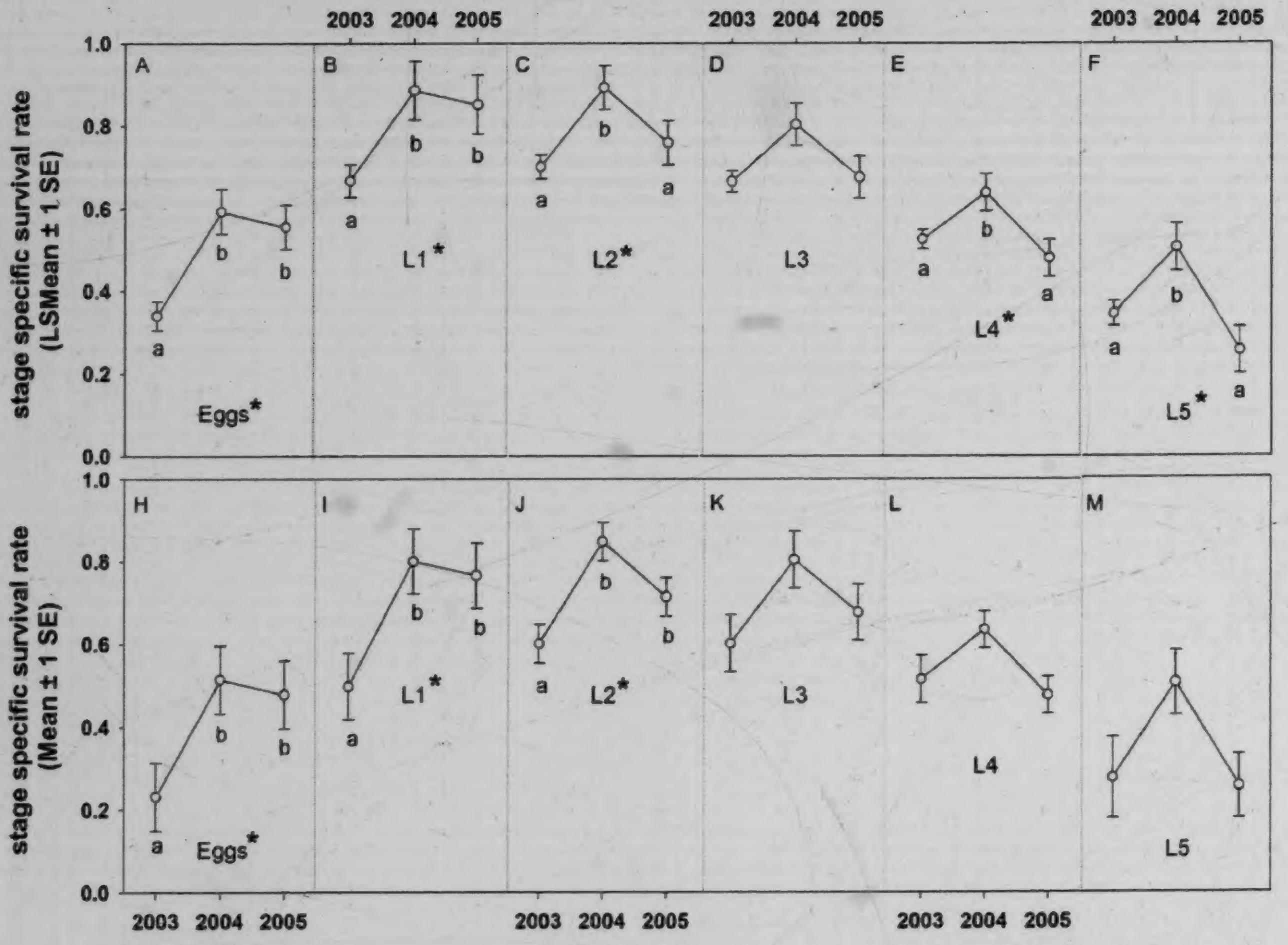

stage specific survival rate (LSMean ± 1 SE)
stage specific survival rate (Mean ± 1 SE)
1.0
0.8
0.6
0.4
0.2
0.0
2003
2004
2005
A
B
C
D
E
F
H
I
J
K
L
M
Eggs*
L1*
L2*
L3
L4*
L5*
L4
L5
a
b

Figure 3.8 Duration of *Profenusa thomsoni* egg and larval stages in Anchorage, Alaska, 2003–2005. All values determined using the KNM model for stage frequency data. Panels A-F show least square means determined from the complete data set (eight sites were sampled in 2003, two sites were sampled in 2004 and 2005, least square means are shown to account for the unbalanced design); Panels H-M show means determined from just those sites sampled in all years. Note that y-axes are not equal. In all panels asterisks indicate a significant effect of year on the given parameter for a stage and lowercase letters indicate significantly different means. Contrasts performed using PROC MIXED (SAS Institute Inc. 2000-2004) with year and crown position as modelled as fixed effects and site as a random effect with year versus year orthogonal contrasts after a significant effect was observed. Note that panel G is missing to allow comparison between this figure and Figure 3.9.

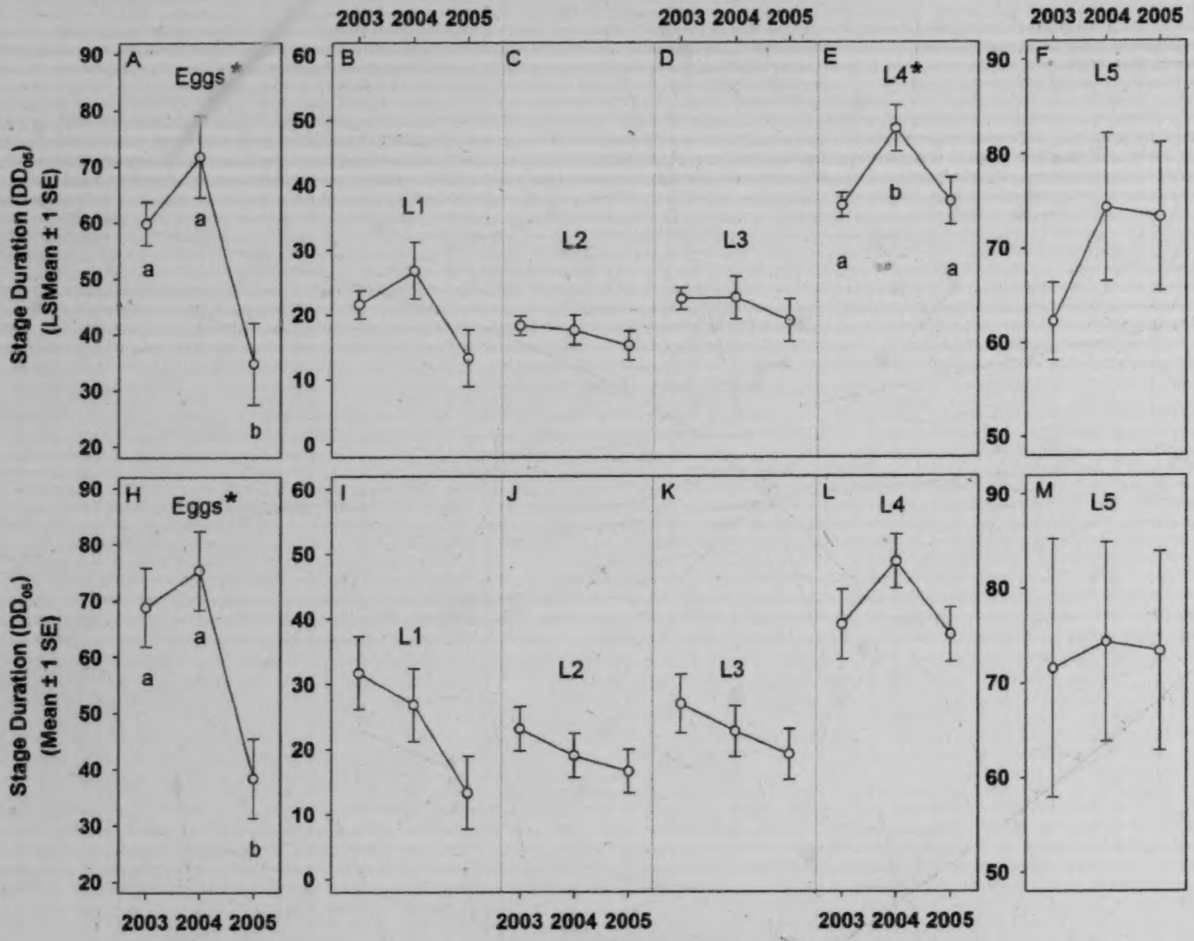
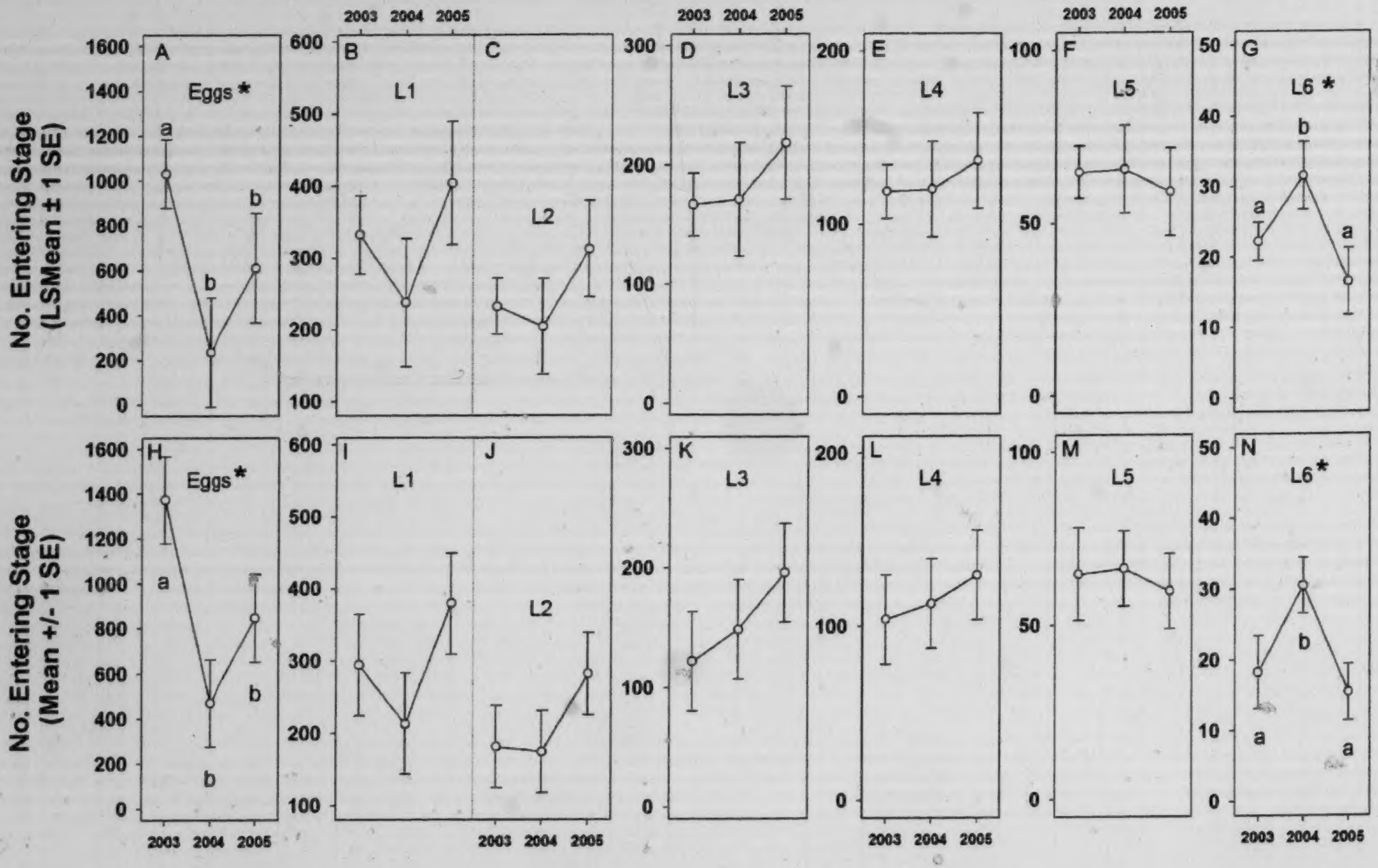

Stage Duration (DD_{05})
(LSMean ± 1 SE)
Stage Duration (DD_{05})
(Mean ± 1 SE)
A
Eggs*
a
a
b
B
L1
C
L2
D
L3
E
L4*
a
b
a
F
L5
H
Eggs*
a
a
b
I
L1
J
L2
K
L3
L
L4
M
L5
90
80
70
60
50
40
30
20
60
50
40
30
20
10
0
2003 2004 2005

Figure 3.9 Number of individuals entering the egg and larval stages of *Profenusa thomsoni* in Anchorage, Alaska from 2003–2005 based on a sample of15 leaves. All values determined using the KNM model for stage frequency data. Panels A-G show least square means determined from the complete data set (eight sites were sampled in 2003, two sites were sampled in 2004 and 2005, least square means are shown to account for the unbalanced design); Panels H-N show means determined from just those sites sampled in all years. Note that y-axes are not equal. In all panels asterisks indicate a significant effect of year on the given parameter for a stage and lowercase letters indicate significantly different means. Contrasts performed using PROC MIXED (SAS Institute Inc. 2000-2004) with year and crown position as modelled as fixed effects and site as a random effect with year versus year orthogonal contrasts after a significant effect was observed.

No. Entering Stage
(LSMean ± 1 SE)
No. Entering Stage
(Mean +/- 1 SE)
A
Eggs *
a
b
b
B
L1
C
L2
D
L3
E
L4
F
L5
G
L6 *
a
b
a
H
Eggs *
a
b
b
I
L1
J
L2
K
L3
L
L4
M
L5
N
L6 *
a
b
a
1600
1400
1200
1000
800
600
400
200
0
600
500
400
300
200
100
300
200
100
0
200
100
0
100
50
0
50
40
30
20
10
0
2003
2004
2005

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Chapter 4. Biological control of *Profenusa thomsoni* in Alaska

Introduction

More than 400 species of invasive alien insects (IAS) attack woody plants in North America (Langor *et al.* 2008; Mattson *et al.* 1994) and 19 IAS are recorded among the 70 most damaging forest insects in Canada and the USA (Liebhold *et al.* 1995). Prevention and early eradication of new infestations are the best ways to manage IAS, but once a new alien has established and spread, the strategies available for control are similar to those used for native pest species (Liebhold *et al.* 1995), with one exception. As IAS are often introduced without their native complement of natural enemies they are reasonable subjects for classical biological control programs that seek to establish some level of permanent regulation by restoring a 'natural' balance between an IAS and its natural enemies. These programs are cost efficient in the long run and have resulted in some level of control in about 50% of attempts against forest pests (Liebhold *et al.* 1995).

Classical biological control has been employed against several important forest sawfly (Hymenoptera: Tenthredinidae) pests in North America, e.g. *Olesicampe geniculatae* Quednau and Lim against the mountain ash sawfly (*Pristophora geniculata* [Hartig]) (e.g., Quednau 1990), *Mesioleius tenthredinis* Morl. against the larch sawfly (*Pristiphora erichsonii* [Hartig]) (Smith 1993) and *Lathrolestes nigricollis* (Thomson) against the birch leafminer (*Fenusa pumila* Leach) (e.g., Fuester *et al.* 1984). In a variation on classical biological control, populations of another IAS, the ambermarked birch leafminer (*Profenusa thomsoni* [Konow], Hymenoptera: Tenthredinidae), a birch (*Betula*) defoliator, have been reduced and held at low population size in and around Edmonton, Alberta (AB) by a putatively native parasitoid, *Lathrolestes luteolator*[1] Gravenhorst (Hymenoptera:

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[1] A recent taxonomic study suggests this species may not be *Lathrolestes luteolator* (A Bennett, Canadian National Collection of Insects, pers. comm.). However, I use the *L. luteolator* designation in this chapter as it is the current published name for the species attacking *Profenusa thomsoni* (Barron 1994)

Ichneumonidae) that adopted *P. thomsoni* locally (Barron 1994; Digweed 1998; Digweed *et al.* 2003) and possibly elsewhere in Canada (Martin 1960).

Profenusa thomsoni is a native of Europe, central Asia, and Japan, and was first recorded from North America in the eastern United States in the 1920s (Smith 1971). By the 1940s *P. thomsoni* was widespread in eastern Canada (Martin 1960) and occasionally reached outbreak levels in Ontario (Haack and Mattson 1993). It arrived in AB in the 1960s (Drouin and Wong 1984) where it contributed to significant defoliation of urban birch every year until the early 1990s (Digweed *et al.* 1997; Drouin and Wong 1984; Langor *et al.* 2002). *Profenusa thomsoni* was introduced to the Alaska (AK) panhandle region sometime during the late 1980s, although it was at first misidentified as *Fenusa pusilla* (now *Fenusa pumila* Leach) (USDA Forest Service 1991; Snyder *et al.* 2007). By 1996 *P. thomsoni* was positively identified in the Anchorage area and near Fairbanks (Snyder *et al.* 2007). When the first extensive surveys were made in 2004 *P. thomsoni* was resident throughout the southeast region of the state, defoliating as many as 24 000 ha every year, with its range still expanding (Snyder *et al.* 2007).

The impact of outbreak populations of *P. thomsoni* on tree health and aesthetic value can be severe (Hoch *et al.* 2000; Snyder *et al.* 2007), but control options are limited. Systemic insecticides are available and are occasionally used in urban settings (Chapter 1; Drouin and Wong 1984) but can be difficult and expensive to apply. Native natural enemies, such as ants and spiders, attack *P. thomsoni* larvae but do not exert control over populations (Digweed 1998; Martin 1960; Pezzolesi and Hagar 1994). Therefore, in 2004 a classical biological control program was initiated to introduce *L. luteolator* from Canada to AK to suppress *P. thomsoni*.

Herein I document an attempt to establish *L. luteolator* in AK wherein I describe the parasitoid mass rearing method, parasitoid releases between 2004 and 2007, and the results of establishment surveys. I compare estimation methods for predicting percent parasitism and discuss the implications for obtaining a large population of biological control agents for release. Finally, I compare the probability of establishment in this study to what was achieved in previous releases of *Lathrolestes* spp.

Methods

There are no published techniques for rearing *L. luteolator*. Therefore the methods I used are based on those developed for other *Lathrolestes* spp. and subsequently modified to help improve rearing success (see Appendix 4). Throughout this chapter sawfly and parasitoid generations are referred to by their year of collection; the number of parasitoids released is given for the year the release was made. For example: *L. luteolator* adults from the 2003 generation were released in 2004 as adult parasitoids.

Target species and Biological Control Agent

Profenusa thomsoni

This species is a univoltine, parthenogenic leafmining sawfly that attacks most birch species (Digweed *et al.* 1997; Hoch *et al.* 2000; Martin 1960; Peirson 1929). Larval feeding results in blotch shaped mines on the upper surface of infested leaves, these mines eventually turn brown thereby decreasing the tree's aesthetic value and photosynthetic capacity. In Anchorage, adults emerge in mid-to-late June (c. 450 degree days above 5°C, pers. obs.) and immediately start laying eggs under the upper epidermis of fully expanded birch leaves. These eggs hatch in 7–12 days and the emerging larvae immediately begins feeding. Larvae spend five instars feeding on the palisade mesophyll layer of the host leaf. After molting to the sixth larval instar the leafminer cuts a hole in the leaf, drops to the ground and enters the soil where it constructs a pupal cell by webbing together soil particles. The leafminer overwinters as an eonymph, pupating shortly before emergence (Martin 1960).

Lathrolestes luteolator

This species is a koinobiont endoparasitoid that lays eggs in developing *P. thomsoni* larvae. Adult females will attack larvae as young as the second instar depositing a single egg in the body cavity (usually in the abdomen) of the host. The egg hatches shortly after being deposited in the host, but feeding does not begin until the leafminer has entered the soil and constructed a pupal cell. After consuming its host, the parasitoid spins a cocoon within its host's pupal cell where it overwinters as a late instar larva. The parasitoid pupates shortly before emergence which seems to coincide with the appearance of early instar *P. thomsoni* larvae, but males usually emerge one to two days before females (pers. obs.).

Collection Sites

Adult *L. luteolator* and *P. thomsoni* larvae were collected from four sites with large *L. luteolator* populations: Fort Smith, Northwest Territories (NT), Hay River, NT, Edson, AB and one park in Edmonton, AB. All sites were urban plantings of 2–60+ native (*Betula papyrifera* Marsh or *B. neoalaskana* Sarg.) or ornamental birch (*B. pendula* Roth.) growing in lawns. Collections were made between late July and early August of 2003–2007, although not all sites were used in all years (Table 4.1).

Collection and Rearing

Rearing methods were adapted from Fuester *et al* (1984). Leaves containing fourth, fifth and sixth instar *P. thomsoni* larvae were plucked from trees, packed in 1 L Ziploc plastic bags (c. 500 leaves per bag) and held on ice for transport to Edmonton, AB. In Edmonton, leaves were placed in 2.5cm square nylon mesh bags that were suspended inside large (60–70L), air-filled, clear plastic bags and hung from the ceiling of a rearing room. This method maintained leaves in good condition, thus encouraging larvae to complete development in their leaf before emergence, and provided a simple way to collect and count emerging larvae. As larvae emerged from leaves in the mesh bag they dropped to the bottom of the plastic bag where they were collected and counted. Larvae were removed from the plastic bags every 12–24 hours and placed into a clear rearing tub 30cm × 45cm × 15cm (L×W×D). Each tub contained 8–10cm of well-moistened sterile rearing medium (three parts sterilized commercial potting soil to one part sand). Small screened holes in the bottom of the tub allowed drainage of excess moisture and larger screened holes in the lid allowed ventilation. As there is no non-destructive method to distinguish parasitized larvae from unparasitized larvae, all insects that emerged were placed into rearing. Larvae were introduced to the rearing tubs by scattering them over the surface of the rearing medium and allowing them to burrow in through cracks and crevices. The number of larvae placed in a tub was recorded but any larvae remaining on the surface 12 hours after being introduced were removed and not included in the final count. Larvae collected from the same collection site were placed in the same rearing tub, with each tub containing between 150 and 1150 larvae (most tubs had 700–1000 larvae). All rearing tubs were stored at ambient outdoor temperature in an insectary until late September and were watered whenever the upper surface of the rearing medium became dry.

In late September, all rearing tubs were removed from the insectary and taken outdoors to a sheltered location, buried in soil up to the level of the lid and then covered with leaves or wood chips. In late May or early June of the following year the rearing tubs were removed from the soil, the outside washed, and packaged for shipment. For shipment, each rearing tub was sealed inside a plastic bag and then placed in a sealed shipping tub which in turn was placed into a cardboard box. Packing material (Styrofoam chips or wadded newspaper) was placed in the cardboard box, within the shipping tub and between the surface of the rearing medium and the lid of the rearing tub to prevent damage during shipment. All tubs were transported to Anchorage, AK either by air as checked luggage aboard a commercial airliner (2004–2006) or overland in a van (2007).

Parasitism was assessed before emergence began using independent samples taken from individual rearing tubs. A 40–300mL volume of rearing medium was removed from 1–5 randomly chosen rearing tubs each year. Tubs were sampled once only and the volume of rearing medium removed was replaced with an equal volume of fresh medium. Depending on the volume of the sample and the number of pupal cells recovered either all pupal cells in a sample were collected or the first 30–50 cells found were collected. For those samples where only 30–50 pupal cells were collected the remaining, un-searched volume of rearing medium was returned to its original tub. All pupal cells recovered were dissected and their contents determined.

All rearings in AK occurred outdoors in emergence cages similar to the mating cages used by Guevremont and Quednau (1977) and Fuester *et al.* (1984). The sides and top of each rearing cage were screened with fine (no-seeum) mesh and the front and back were constructed from 0.6 cm thick plywood with a mesh sleeve in the centre of one side that permitted access to the interior. Each open-bottomed 0.5 m × 0.4 m × 0.6 m rearing cage enclosed one rearing tub. The lid of each rearing tub was used as a sunshade and as a place for newly emerged insects to rest and avoid direct sunlight by placing it such that a third to a half of the surface of the rearing medium was exposed. Each cage was provisioned with sugar water or honey (refreshed every 3–4 days) and water (refreshed daily). The rearing medium was kept moist by occasional watering and by rainfall. A large outer screen tent, used as a sunshade, enclosed all the rearing cages in 2004 but was not used in subsequent years.

Parasitoids were reared at four sites in Anchorage between 2004 and 2007. The first site, used in 2004 and 2005, was located on the grounds of the Municipality of Anchorage greenhouses. This site was well sheltered from wind and was sun-exposed for most of the day. The soil was hard packed and there were a few overhanging poplar (*Populus* sp.) and spruce (*Picea* sp.). The other three rearing sites, used in 2006 and 2007, were located in a flower bed, a derelict garden plot and a lawn, all on private property. The soil at these sites was looser and there was less sun exposure than at the first site.

Cages were checked for emergence every 24–48 h from 15 June until emergence ceased (c. mid-August) in all years. All *P. thomsoni* and *L. luteolator* adults visible inside each rearing cage were removed using an aspirator, sexed and counted. Adult *P. thomsoni* were killed and preserved, *L. luteolator* dead at the time of collection and those that died in captivity were also preserved. Live adult *L. luteolator* obtained from the rearing cages were released 2–120 hours after collection. Insects held for more than 24 hours were kept in small 150–350mL containers or rearing cages (30cm × 30cm × 30cm or 15cm × 15cm × 15cm) at 4ºC. All containers and rearing cages were provided with water and honey, or a sugar solution. During the holding period the parasitoids were occasionally removed from 4ºC and held at 20ºC for up to 2 hours to allow them to feed and mate, after which time they were returned to 4ºC.

Adult Collections

In order to supplement the population of parasitoids obtained from rearing of host material, additional adult *L. luteolator* were collected from Hay River, NWT, Edmonton AB and Edson, AB (Table 4.1). Free-flying adults were aspirated from birch and placed in containers (as above) containing water and sugar sources then flown to AK as carry-on luggage aboard a commercial airliner within 48 hours of collection. While in transit the containers were carried in a picnic cooler with an ice pack. The number of parasitoids that survived the trip and were released is reported in Table 4.2. Those that died before release were preserved.

Parasitoid releases

Parasitoids were either allowed to freely disperse within stands of infested birch (Fuester *et al.* 1984) or briefly caged on branches prior to being released. Parasitoids that were caged were placed in mating pairs for 24–48 hours in fine mesh cages enclosing 10–15 *P. thomsoni* infested leaves on individual, living birch trees then allowed to disperse. Both open-air releases (Environment Canada- Ecoaction 2003; Fuester *et al.* 1984; Guèvremont and Quednau 1977, 1978; van Driesche *et al.* 1997; Vincent *et al.* 2001) and cages (Guèvremont and Quednau 1977, 1978; Raske and Jones 1975) have been used to establish *Lathrolestes* spp. elsewhere. All releases in 2004 and 2005 were of the open-air type but a combination of both methods was used in subsequent years. Release sites were selected on an *ad hoc* basis using the following criteria: the age of leafminers in leaves (presence of late-instar larvae preferred); the availability healthy trees with abundant foliage and low crowns to allow observation of parasitoid activity; no history of insecticide treatments to trees or surrounding area and no likelihood of future treatment; high likelihood that the release sites would remain undisturbed and intact for a sufficient number of years to allow a population of parasitoids to establish, increase and disperse.

Monitoring Establishment

Adult trapping, larval sampling and direct observation were used to assess establishment of *L. luteolator* in AK. Monitoring at each release site commenced one to two years after the first releases were made. Adult parasitoids were trapped using 3–5 yellow sticky cards (Pherotech, Delta, British Columbia) hung in the lower crown (approx 1.5 m above ground) (Digweed *et al.* 2003; McQueen 1996) that were checked daily. Larval samples were also collected and assessed for evidence of *L. luteolator* parasitism. Approximately 100 infested leaves were collected from 1–5 trees at five-day intervals between 5 and 29 August 2006. At each sampling date 150 fourth, fifth and sixth instar *P. thomsoni* larvae from these leaves were dissected to ascertain the presence of parasitoid eggs and larvae (*sensu* Fuester *et al.* 1984). Lastly, observers visited each release site during the period of expected flight activity of *L. luteolator* and visually examined trees. Any suspect adult *L. luteolator* observed during these searches were aspirated, preserved and identified by Scott Digweed of the Canadian Forest Service, Northern Forestry Centre or Andrew Bennett of the Canadian National Collection of Insects using reference specimens and published keys (Barron 1994).

Statistical Analysis

Percent emergence (defined as the total number of insects emerging from a rearing tub divided by the total number of *P. thomsoni* larvae placed in the same tub) and realized percent parasitism (defined as the number of adult parasitoids emerging from a rearing tub divided by the total number of insects emerging from the same rearing tub) were calculated for all rearing tubs and used as the raw data for analyses ANOVA. Predicted percent parasitism (i.e., the number of *L. luteolator* larvae and pupae divided by the total number of live insects found) was determined from the pre-emergence samples and correlated against realized percent parasitism to evaluate the predictive ability of pre-emergence estimates. The absolute difference between realized percent parasitism and predicted percent parasitism was determined and then correlated with the number of pupal cells in the pre-emergence sample to derive the sample size necessary to generate reliable estimates of percent parasitism.

All ANOVA were performed in SAS v 9.0 using PROC GLM (SAS Institute Inc. 2000-2004), with single rearing tubs treated as a replicates. When necessary, data were arcsine-square root transformed to meet the assumptions of homogeneity of variance and normality of residuals but untransformed data are presented. Furthermore, full models were considered in all ANOVA but because no significant interactions were detected I only report main effects. Correlation coefficients were determined using PROC CORR (SAS Institute Inc. 2000-2004).

Results

Rearing

Over the four years of rearing, 2301 *L. luteolator* adults (1176F:1125M) and 3705 *P. thomsoni* adults emerged out of a total population of 38,361 larvae (Table 4.3). Percent emergence of insects (sawflies + parasitoids) was low (mean, all years combined = 12.7%) and did not differ among collection sites for any generation (p = 0.2–0.8, Figure 4.1).

Realized percent parasitism of populations in Hay River and Fort Smith did not differ significantly at either site between 2003 and 2005 ($p = 0.08$; Figure 4.2), but was significantly higher in 2006 at both sites (ANOVA, Hay River and Fort Smith rates only, *a priori* orthogonal contrast of 2003–2005 to 2006: F = 35.50, df = 1, $p < 0.01$; Figure 4.2). The Hay River population experienced higher percent parasitism than the Fort Smith population and this difference was significant for all but the 2006 generation (F = 22.23, df = 3, $p < 0.05$; Figure 4.2).

Parasitoid Releases and Adult Collections

A total of 2685 (1155F:1530M) *L. luteolator* were released in AK between 2004 and 2007, 1758 (924F:834M) were reared and 927 (231F:696M) were collected as live adults (Table 4.2). Insects were released at four sites in Anchorage; 215 (118F:97M) in 2004 and 2005 at Balto Seppela Park (BS, Figure 3.1), 418 (122F:296M) in 2006 and 705 (188F:517M) in 2007 at two sites on the campus of the University of Alaska – Anchorage and 974 (456F:518M) in 2007 at Javier de la Vega Park (JV, Figure 3.1). Insects were also released at two sites in the Interior; 2 (2F:0M) in 2004 on Eielson Air Force Base and 241 (179F:62M) in 2007 in the city of Fairbanks; and at one site on the Kenai Peninsula; 130 (90F:40M) in 2007 in the town of Soldotna (Table 4.2).

Monitoring Establishment

Two adult female *L. luteolator* were collected from birch trees at the 2006 University of Alaska release site on 31 July 2007. The yellow sticky cards deployed at Balto Seppela Park during July and August 2006 failed to recover any adult *L. luteolator*. However, 6–17% of *P. thomsoni* larvae collected at the same site in August 2006 bore melanized eggs and early instar parasitoid larvae in their body cavities (Figure 4.4). These larvae could not be conclusively identified but were morphologically similar to known *L. luteolator* larvae dissected from *P. thomsoni*, and similar to descriptions of larvae of other *Lathrolestes* species (e.g. Eichhorn and Pschorn-Walker 1973).

Estimates of expected percent parasitism did not predict realized percentage parasitism. There was no significant correlation between the estimated percent parasitism from pre-emergence soil samples and the observed percent parasitism (Pearson's r = -0.115, *p* = 0.75; Figure 4.4A). However, there was a weak, negative correlation between the error in percent parasitism estimates and sample size (Pearson's r = -0.590, *p* = 0.06; Figure 4.4B) with a smaller estimated error from larger samples, although this is expected when sampling a binomially distributed population (Zar 1999). All pre-emergence soil samples contained fewer than fifty specimens except one sample containing 167 specimens. The large sample was not used in these analyses because it was at least three times larger than all other samples and would exert a significant effect on any correlation co-efficient.

Discussion

Between 2004 and 2006 a total of 2685 *L. luteolator* were released at seven sites in AK. The major factor constraining the number of *L. luteolator* available for release was the poor emergence from rearing tubs. The rearing protocol used here for *L. luteolator* was based on methods used previously to rear *L. nigricollis* (Fuester *et al.* 1984; McQueen 1996) but were less successful for *L. luteolator* (Appendix 4).

Rearing, Releases and Establishment

It is possible that *L. luteolator* has established at least one population in Anchorage. The two female specimens collected in 2007 from the 2006 release site on the University of Alaska–Anchorage campus suggest there is a reproducing population at that site. However, *L. luteolator* were released in 2007 at a second site on the UAA campus 800 m from the 2006 release site 8 days prior to collection of the specimens (Table 4.2). Thus it is possible that the parasitoids recovered in 2007 at the 2006 site originated from that release. As for the other, pre-2007 release sites; The 2 *L. luteolator* released on Eielson Air Force Base in 2004 are well below the threshold of insects necessary to establish a viable population of biological control agents (Hopper and Roush 1993) and thus are unlikely to have resulted in a permanent population. In Anchorage no adults were captured on sticky cards hung in Balto Seppela park in 2006 but a significant percentage of larval *P. thomsoni* at that site were parasitized (Figure 4.4). However, *P. thomsoni* taken from another site in Anchorage where *L. luteolator* had not been released were also been found to contain similar parasitoid larvae (Anna Soper, University of Massachusetts-Amherst, pers. comm.). This observation suggests two scenarios; either *L. luteolator* was established and distributed in Anchorage prior to 2006 or that some other parasitoid is attacking *P. thomsoni* in AK.

If *L. luteolator* was established in Anchorage prior to 2006 these populations could only have resulted from the rapid spread of individuals released at Balto Seppela Park in 2004 and 2005. A survey in 2002 did not detect *L. luteolator* (Daryl Williams, Canadian Forest Service, unpublished) nor did I encounter it during intensive sampling between 2003 and 2005 at eight other sites in Anchorage (Chapter 3), nor have I observed adults attacking *P. thomsoni* larvae or collected them from emergence traps set below infested birch trees. The dispersal rate of *L. luteolator* has not been investigated, however Langor *et al.* (2000) found that *L. nigricollis* dispersed at least 1 Km three years after release and at least 13 Km six years after release. One kilometer is approximately the same distance between Balto Seppela Park and the site at which parasitized larvae were found in 2006. Alternately, and probably more likely, is that there is one or more unknown parasitoids that are attempting to use *P. thomsoni* as a host. There is no evidence that this parasitoid is successfully reproducing and it does not appear to be exhibiting any control of *P. thomsoni* in AK (Snyder *et al.* 2007) which serves to highlight the need to continue introductions of *L. luteolator*.

Likelihood of establishing *L. luteolator*

The successful introduction of a biological control agent is often referred to as a 'numbers game'; or the probability of establishment should increase with the number of insects released. General thresholds are proposed beyond which the probability of establishment is thought to be high (Hopper and Roush 1993), but it may not be possible to collect or rear enough individuals to meet these values, as was seen here. However, examining successful introductions of the same or related species that also released small numbers may give insight into what population size, for that group, is associated with establishment. Populations of *L. nigricollis* were established in *F. pumila* populations following releases of as few as 97 parasitoids in a single year (Fuester *et al.* 1984; Appendix 5) and *Lathrolestes ensator* (Brauns) was established in *Hoplocampa testudinea* Klug (Hymenoptera: Tenthredinidae) populations in Quebec following releases of 513 parasitoids over four years (but with 65% of the total release the year immediately prior to the first recovery) (Vincent *et al.* 2001). Furthermore, NT populations of *L. luteolator* used in this study may have been established via the introduction of a few thousand *P. thomsoni* infested leaves (Environment Canada- Ecoaction 2003), which given typical parasitism rates in source populations could mean that fewer than 250 parasitoids were introduced. More recently *L. luteolator* was established (or re-established) in Yellowknife NT from the introduction of 205 parasitoids over three years, only 69 of which were female (Scott Digweed unpublished; also see Appendix 5). Thus the numbers of insects released in this study are on par with previous successful introductions (Appendix 5), suggesting sufficient numbers have been released to give a good probability of establishment.

Potential Barriers to Establishment

While the probability of establishing *L. luteolator* is encouraging it is important to acknowledge potential barriers. Newly introduced biological control agents face an increased risk of extinction because of low genetic diversity (Drake and Lodge 2006; Sakai *et al.* 2001). Genetic diversity in the population of parasitoids released in Anchorage was maximized by collecting large numbers of parasitized hosts from multiple sites separated by a minimum of 195 Km. Hopper and Roush (1993) suggest a threshold of 1000 parasitized hosts should be collected to ensure that the genetic diversity of the source population is reflected in the newly-established population. Based on the realized percent parasitism observed in this study for the four collection sites, well over 1000 parasitized hosts were collected for the 2006 generation and almost as many in the 2003–2005 generations. Of course, this calculation assumes that there is no differential mortality of either species that would result in the realized percent parasitism not reflecting the 'true' value. Samples of *P. thomsoni* and *L.. luteolator* pupae taken from the rearing tubs after emergence had ceased did not detect any such differential mortality (Appendix 4). Therefore it is reasonable to assume that the realized percent parasitism is an accurate estimate and, by extension, a sufficient number of hosts were collected to maintain a high level of genetic diversity.

Estimating percent parasitism

Obtaining accurate estimates of percent parasitism is important when trying to predict population dynamics of hosts and parasitoids. Besides direct effects (i.e., consuming a host), parasitoids can have other lethal effects that do not result in the production of a new offspring, but do kill a host (i.e., host feeding, host paralysis) (van Driesche 1983). Thus the cumulative impact of parasitoids on a population of hosts can be greater than is reflected by percent parasitism. Furthermore, estimates of percent parasitism can be influenced by host and parasitoid phenologies (van Driesche 1983) potentially giving estimates that are not a true reflection of the impact of the parasitoid on the host population. By contrast, when attempting to introduce a biological control agent it is the number of parasitoids that can be produced that is the most important measure as this dictates the size of the population that can be released and, possibly, the number of places where populations can be established. Obtaining accurate estimates of the number of parasitoids can be difficult though, particularly for koinobiont parasitoids like *L. luteolator* where parasitism is not easily observed. This was exemplified in this study where the best estimates of percent parasitism came from large samples of overwintering hosts. Sampling large numbers of insects does not pose a problem when emergence is expected to be high as many insects can be destructively sampled without fear of reducing the population of control agents available for release. However, when emergence is low the need to estimate percent parasitism must be balanced by the need to maximize the population of agents for release.

Conclusions

This study successfully introduced *L. luteolator* to Anchorage, AK in numbers consistent with other biological control efforts that have resulted in establishment. However, at the time of this thesis, it remains uncertain whether the parasitoid has been established in sufficient numbers to be considered a viable, self-sustaining population.

Table 4.1 Sites and dates of collection of *Profenusa thomsoni* larvae and *Lathrolestes luteolator* adults.

	Collection site				Stage Collected*	Year				
Province/ Territory	Name	Lat.	Long.	Description		2003	2004	2005	2006	2007†
Alberta	Edmonton	53.443	-113.503	*B. pendula*, public park	Larvae				7–30.vii	27.vii–11.viii
					Adults				20.vii	19–21.vii
	Edson	53.573	-116.460	*B. neoalaskana*, cemetery	Larvae				24–30.vii	
					Adults				19.vii	
Northwest Territories	Fort Smith	60.013	-112.112	*B. papyrifera* & *B. neoalaskana*, Hospital, town hall	Larvae	28.vii–16.viii	6–24.viii	11–21.viii	17–30.vii	27.vii–6.viii
					Adults					
	Hay River	60.818	-115.784	*B. papyrifera* & *B. neoalaskana* Church grounds, private property	Larvae	1–15.viii	6–16.viii	4–19.viii	17–30.vii	27.vii–11.viii
					Adults			4.viii	31.vii	19–20.vii

* 'Larvae' denotes fourth, fifth and sixth instar *Profenusa thomsoni* larvae; 'Adults' denotes *Lathrolestes luteolator* adults.

† Larvae collected in 2007 are not part of this thesis.

Table 4.2 Release sites and number of *Lathrolestes luteolator* released in Alaska 2004–2007.

Release site			Date(s)	Total *L. luteolator* released*		Source†
Name	Lat.	Long.		Females	Males	
Anchorage						
Balto Seppela Park	61.190	-149.943	7.vii-9.viii.2004	42	15	Reared
			2 .vii-10.viii.2005	71	75	Reared
			6.viii.2005	5	7	Collected
University of Alaska site 1	61.189	-149.829	13.vii-9.viii.2006	67	96	Reared
			21.vii.2006	31	157	Collected
			1.viii.2006	24	43	Collected
University of Alaska site 2	61.189	-149.829	23.vii.2007	17	28	Reared
			23.vii.2007	171	489	Collected
Javier Vega Park	61.168	-149.920	27.vii.2007	122	219	Reared
			31.vii.2007	112	165	Reared
			1.viii.2007	72	40	Reared
			3.viii.2007	88	78	Reared
			14.viii.2007	50	10	Reared
			20.viii.2007	12	6	Reared
				884	**1428**	Anchorage Grand Total
Interior						
Eielson Air Force Base	64.674	-147.073	15.vii 2004	2	0	Reared
Fairbanks	64.845	-147.721	10.viii.2007	179	62	Reared
				181	**62**	Interior Grand total
Kenai Peninsula						
Soldatna	60.498	-151.090	6.viii.2007	90	40	Reared
				1155	**1530**	Grand total

* Releases made in 2007 are attributed to Anna Soper, University of Massachusetts-Amherst.

† 'Reared' denotes adults that emerged from rearing tubs in Alaska; 'Collected' denotes adults collected from trees in Canada and shipped to Alaska

Table 4.3 Total *Profenusa thomsoni* larvae reared and total number of adult *P. thomsoni* and *Lathrolestes luteolator* emerged by generation and source

Generation	**Collection site**	***P. thomsoni* larvae reared**	**Total emergence**			
			P. thomsoni	Female *L. luteolator*	Male *L. luteolator*	
2003	Fort Smith	3434	273	18	21	
	Hay River	3255	320	75	61	
		6689	**593**	**93**	**82**	Sub total
2004	Fort Smith	1602	216	10	16	
	Hay River	3578	302	96	104	
		5180	**518**	**106**	**120**	Sub total
2005	Fort Smith	2926	421	6	16	
	Hay River	3105	535	86	111	
		6031	**956**	**92**	**127**	Sub total
2006	Fort Smith	2297	22	39	58	
	Hay River	3795	60	232	197	
	Hay River*	2986	35	52	69	
	Edmonton	4918	341	155	126	
	Edson	6465	69	407	373	
		20461	**1638**	**885**	**823**	Sub total
		38361	**3705**	**1176**	**1152**	Grand total

* larvae in outdoor overwintering plot. See methods for details

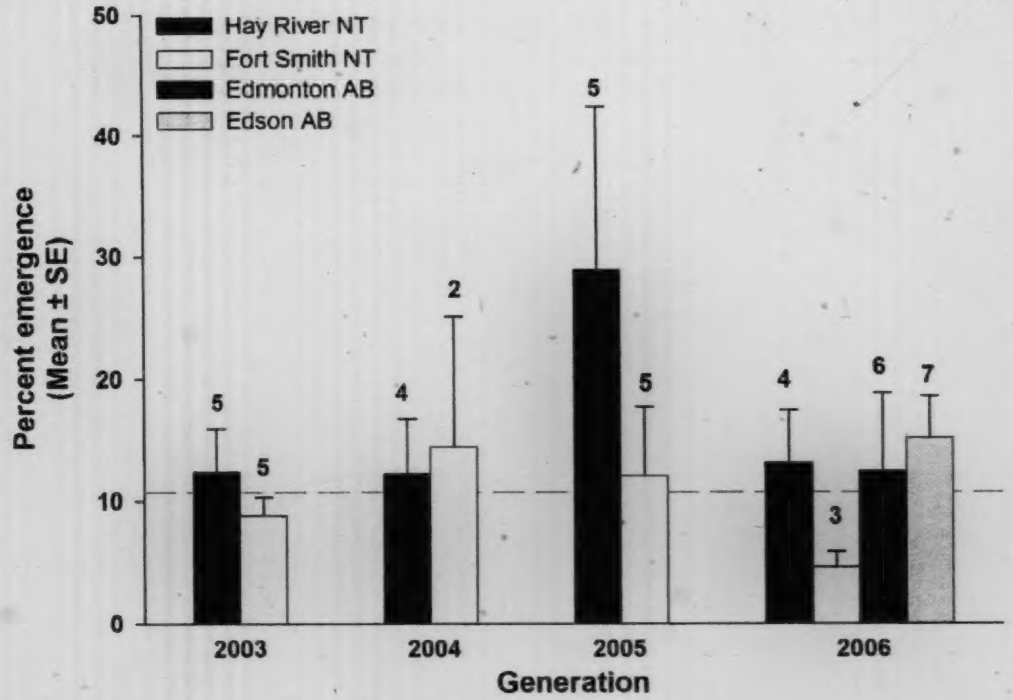

Figure 4.1 Mean percent emergence (± SE) of *Profenusa thomsoni* and *Lathrolestes luteolator* from rearing tubs by generation (= year of collection) and source. Means are not significantly different among sites for each generation. Dashed line indicates overall mean for all generations combined. Numbers above standard error bars indicate sample size (number of rearing tubs).

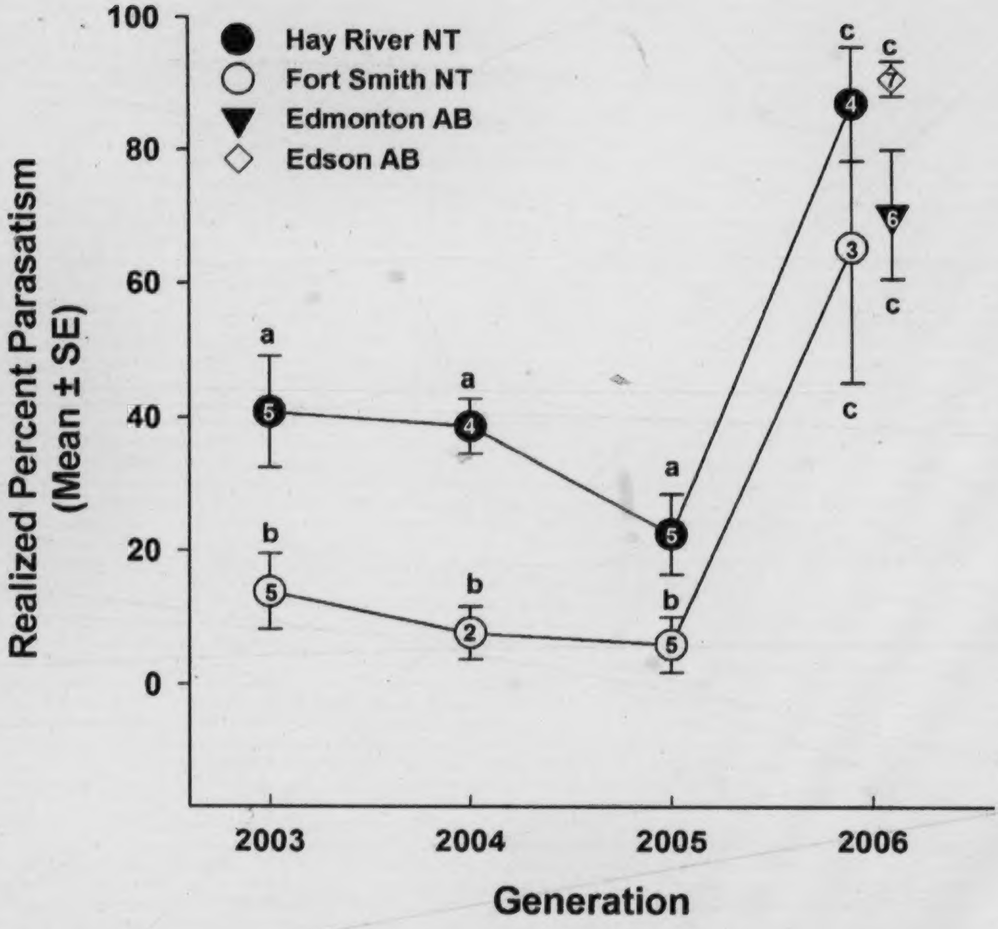

Figure 4.2 Mean realized percent parasitism (± SE) of *Profenusa thomsoni* by *Lathrolestes luteolator* by generation and source. Numbers within symbols represent sample size (number of rearing tubs). Means with the same letter are not significantly different.

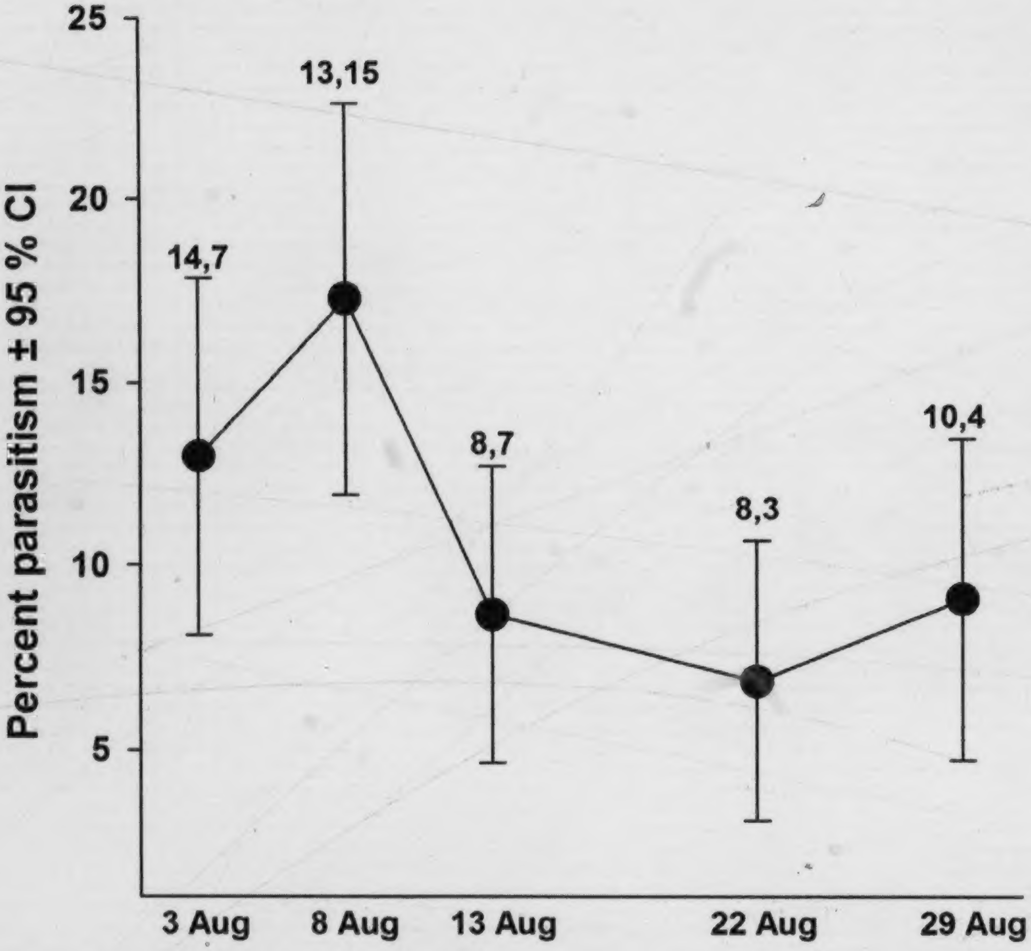

Figure 4.3 Trend in percent parasitism of *Profenusa thomsoni* larvae collected from the first release site (Balto Seppela Park) in 2006. Values above each point give the number of melanized parasitoid eggs and the number of parasitoid larvae collected at each sample date.

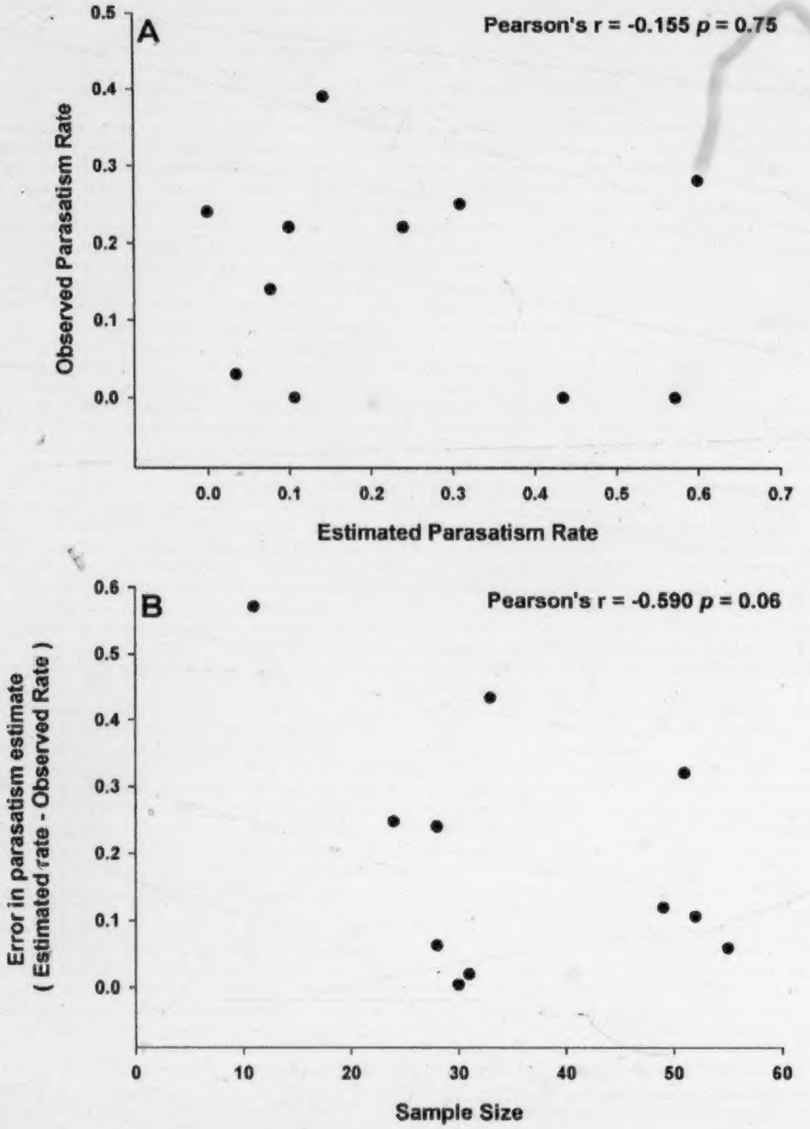

Figure 4.4 Correlation plots of (A) Estimated and Observed parasitism of *Profenusa thomsoni* by *Lathrolestes luteolator* and (B) Sample size versus the error in estimate rates

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Chapter 5. General Discussion

I studied *Profenusa thomsoni* (Konow) (Hymenoptera: Tenthredinidae) at a continental scale to gain insight into its dispersal history, and at the local scale in Anchorage, Alaska (AK) to determine what factors influence its population dynamics. The foregoing chapters show that populations of *P. thomsoni* in North America originated from either one introduction or multiple introductions from the same geographic range as defined by mitochondrial haplotypes (Chapter 2).

In Chapter 3 I conducted a life system analysis of egg and larval *P. thomsoni* in urban birch (*Betula* L.) forests in AK. Through this work I demonstrated that larval survivorship is high and that overall production of offspring is likely at a maximum. I further demonstrated that *P. thomsoni* in AK are not suppressed by parasitoids or natural enemies. Instead, most mortality can be attributed to resource limitation and consequent intraspecific competition within leaves.

In response to local concerns about aesthetic damage to urban birch in AK, I initiated a suppression program *via* the introduction of *Lathrolestes luteolator*[1] Gravenhorst (Hymenoptera: Ichneumonidae) and monitored the early stages of its establishment (Chapter 4). As part of this introduction, I developed and tested field-rearing methods for *L. luteolator* that could be used in future efforts against *P. thomsoni* or in the production of other koniobiont endoparasitoids that attack Tenthredinid sawflies (Appendix 4). These rearing methods could have practical application in future biological programs or in empirical studies of host/parasitoid ecology.

My results established that two analytical techniques, stage-frequency models and classification trees, can be used to monitor shifts in populations of leafminers like *P. thomsoni*. These techniques, as applied in this context for the first time may have general application in the study of insect population dynamics.

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[1] See footnote to Chapter 1 on page 5.

Implications for birch leafminer pest management

The introduction of *L. luteolator* to AK appears to have been successful. However, further monitoring will be required to determine if a population can establish itself and bring *P. thomsoni* populations under control. This particular biological control agent has been released too few times to infer the likelihood of its establishing in a new location (Appendix 5). However, a number of lines of evidence suggest the probability of establishment is high. First, introductions of *L. luteolator* to Yellowknife, Northwest Territories made during the period of this study also appear to have been successful (Appendix 5; Scott Digweed pers. comm.). Second, 41–50% of *Lathrolestes* spp. releases against other Tenthredinids have been successful (Appendix 5) suggesting that as a group, *Lathrolestes* spp. are amenable to relocation and establishment in new areas. Third, Ichneumonid parasitoids can establish viable populations with fewer individuals and with fewer releases than other parasitoid taxa (Hopper and Roush 1993). Fourth, biological control efforts against endophagous herbivorous insects have a good record of success (Smith 1993; but see Stiling 1990).

In natural systems leafmining insects like *P. thomsoni* are often regulated by parasitoids (Hespenheide 1991). In AK, the *P. thomsoni* parasitoid 'niche' is empty; suggesting there will be little completion with *L. luteolator* for hosts. Gross *et al*'s (2006) model of parasitoid establishment predicts that *L. luteolator* should establish in 15–66% of attempts. This model was developed by examining successful biological control introductions and takes into account the host's niche and taxonomic group, the habitat of the affected plant, the severity of damage caused to the plant and its use (e.g., crop plant, horticulture, etc). The model predicts the observed range of successful *Lathrolestes* spp. establishments (see above and Appendix 5) and furthermore suggests that parasitoid introductions in urban habitats against an insect like *P. thomsoni* should have a higher frequency of success (Gross *et al.* 2006). Long-term monitoring of *L. luteolator* in Anchorage will be necessary to determine if my efforts are successful over the long term, but the anecdotal, empirical and model-based evidence presented here suggest that establishment is likely.

Contributions to the understanding of leafminer and parasitoid ecology

The factors that likely influence the population dynamics of *P. thomsoni* populations in Anchorage differed substantially from that observed in other populations of birch leafmining sawflies. I hypothesized that some of these differences are related to the short period this species has been resident in AK (Chapter 3). To evaluate this hypothesis, it will be necessary to re-evaluate the dynamics of *P. thomsoni* populations in AK at regular intervals to quantify changes that may occur, as a number of factors could become important. For instance, birds and other natural enemies may adopt *P. thomsoni* as prey; *L. luteolator* or other parasitoids may become significant mortality factors; other leafminers may invade, increasing competition for limited resources; or exogenous features, such as climate change, could alter the linkage between birch and leafminer phenologies. Evolutionary and behavioural adaptations can be expected to occur in both invasive species and native species that could significantly alter population dynamics (e.g., Elkinton *et al.* 1996). My thesis highlights the importance of studying invasive species at various points along the invasion continuum.

In the course of sampling for this thesis one minor but interesting difference between the Anchorage population and previous outbreaks was noted. Both Martin (1960) and Digweed (1998) observed that all *P. thomsoni* larvae, regardless of stage, would exit a leaf if it became defoliated (100% of green tissue consumed). This behaviour was not observed in the AK where defoliated leaves often contained dead larvae. Abandoning defoliated leaves confers no apparent advantage to young (first to fourth instar) larvae as they are unable to enter a new leaf or construct a pupal cell (Martin 1960). The fact that dead larvae did not abandon leaves in the Anchorage population was somewhat fortunate as these records of mortality formed the basis of the classification tree analysis

(Chapter 3). Although larvae observed in the earlier studies died on the ground (Martin 1960), rather than in the leaves as they did here, their fate was the same. I do not know why the Anchorage population does not exit defoliated leaves but I did note that these leaves were dry and prone to splitting open, spilling their contents. This has the same effect as larvae exiting the leaf and could be misinterpreted as such.

Repeated collection of parasitized *P. thomsoni* from the same geographic locations made it possible to track realized parasitism rates of two natural *P. thomsoni* populations (Hay River and Fort Smith). Prior to this study, the only information about rates of parasitism of *P. thomsoni* by *L. luteolator* was provided by sticky-card and emergence trap data from Edmonton during the 1990s. At that time Digweed *et al.* (2003) showed percent parasitism varied among years: 11% in 1992, 81% in 1994 and 68% in 1995 (Digweed *et al.* 2003). My rearing data showed a similar pattern of variation in realized percent parasitism; rates were static from 2003 through 2005 at c. 20–25% but were significantly higher in 2006 at c. 75% (Figure 4.3). Although this pattern of percent parasitism may be a function of the collection method (see below), the large year-to-year increases in percent parasitism could reflect a numerical response by *L. luteolator* to increasing host density. If so, then data from this study and from the 1990s suggest this response can occur quickly, i.e., over a single year.

In 2006 similar rates of *L. luteolator* parasitism were observed at both Alberta (AB) collection sites and at both collection sites in the Northwest Territories (Figure 4.3). I propose three hypotheses to explain why sites separated by hundreds of kilometres had identical rates of % parasitism.

1) The similar rates of parasitism could have resulted from inadvertent mechanical damage while in transport. All the rearing tubs containing the 2006 generation were trucked to Alaska, rather than being flown as in previous years (Chapter 2). Parasitoid cocoons may be more resistant to mechanical damage

(e.g., from road vibration) than are leafminer pupal cells. If so, shipping could have resulted in disproportionate mortality of *P. thomsoni* and thus more similar levels of parasitism from the four collection sites.

2) The similar rates of parasitism may be an artifact of the collection method. As the primary goal of this project was to collect large numbers of parasitoids there was a distinct bias towards collecting *P. thomsoni* larvae from sites where *L. luteolator* adults were known (or thought) to be abundant. It is possible that collectors in 2006 were more efficient at finding large numbers of parasitized hosts than in previous years. This was the case for at least one collection site in 2006 where the collapse of the *P. thomsoni* population forced us to make collections in areas where *L. luteolator* abundance was likely higher than in previous years (S Digweed pers. comm.). If this hypothesis holds, then it is likely that the estimates of parasitism presented in this thesis do not represent the 'true' percent parasitism in the non-randomly sampled populations (van Driesche 1983; van Driesche *et al.* 1991).

3) If mechanical damage and biased sampling are discarded as explanations for the similar rates of parasitism then one might suggest that *L. luteolator* responds to higher-order processes that synchronize its population dynamics over broad areas. Populations of other insects are known to have synchronized dynamics over similar spatial scales (Liebhold *et al.* 2004) but, to my knowledge, this has not been demonstrated for a parasitoid species. *Lathrolestes luteolator* populations appear to fluctuate in the same way over large spatial scales in a way seemingly independent of the host population (Figure 3.4), thus providing weak support for this hypothesis.

Application of stage structured population models

Stage frequency models have been little used despite potential utility, particularly in the analysis of data describing species for which there is significant overlap of the life stages (summarized in Manly 1990; also Gross *et al.* 2002; Manly 1997;

Munholland and Kalbfeisch 1991; Southwood 1978; Yamamura 1998). Most life table studies adopt a cohort approach (Carey 1993) to evaluate the relative impact of different mortality factors and how they change through time. To do this appropriately requires an entire population be monitored until all individuals have died (Carey 1993; Varley *et al.* 1973). Alternately, and more appropriately for most invertebrate populations, the same population is repeatedly sub-sampled, the developmental stages enumerated and assessed for nascent mortality by rearing these individuals until they die or become adults (e.g., Morris 1960; Royama 2001). Statistical models can then be applied to the resulting data to determine population parameters and the relative importance of the different mortality factors (Royama 1981; Varley *et al.* 1973). However, methodological constraints limit this approach to those species that can be easily sampled and reared.

Alternately, by using the approach I illustrated in Chapter 3 reasonable estimates of population parameters can be developed for many species that cannot be reared or for which resources are too limited to effectively monitor. The application of stage-structured population models may have particular utility in the study of newly-identified invasive alien species, particularly those species for which little is known. These statistical techniques may also be useful for the study of organisms in habitats such as urban environments where it may be impossible to establish large-scale sampling and rearing programs.

Suggestions for future work

In Chapter 2 I demonstrated that North American populations of *P. thomsoni* are genetically uniform. Similar results were shown for populations of *S. betuleti* and *S. vicina*. Future work should expand on the genetic studies of these three species, in particular extending the studies of *Scolioneura* to newly discovered populations in AB and eastern Canada (Chapter 2 Digweed *et al.* submitted). Also *S. vicina* appears to have a high degree of genetic variability across its range in Europe (Chapter 2). This pattern warrants further investigation particularly in light of the recent finding of extensive hybridization in the related group of pine-feeding Diprionid sawflies (Linnen and Farrell 2007). Those authors' results suggest future work should examine the evolutionary history of the birch-feeding sawflies and their relatives to look for similar hybridization events. If hybridizations have occurred the interpretation of results in this thesis would be significantly altered. In particular, *S. vicina* and *S. betuleti* could indeed be separate species as proposed by Altenhofer and Taeger (1998).

I hypothesized in Chapter 3 that competition and resource exhaustion were important factors predicting *P. thomsoni* larval mortality in Alaskan populations (Chapter 3). Future work should test these hypotheses and determine if the patterns suggested in the classification trees reflect actual processes that determine the population size of *P. thomsoni*. It would be interesting to repeat these analyses on other populations of *P. thomsoni* to test the consistency of the mortality factors I identified to predict mortality elsewhere. Furthermore, the classification tree analysis could prove useful in the analysis of other leafminer systems (e.g., Auerbach and Simberloff 1984; Cornelissen and Stiling 2003; Stiling *et al.* 1999; Wilson and Faeth 2001) and help establish if general patterns hold among species. It would be particularly interesting to apply these analyses *post-hoc* to species and systems that have been analyzed using more conventional statistics (e.g., general linear models or regression) to compare the analytical strength among methods. If classification trees generate the 'expected' response in systems that are well understood we would have greater confidence in their application to new systems.

Unanswered question

A potentially important aspect of *P. thomsoni* in AK that was not addressed in this thesis relates to the behaviour of leafminer populations in native forests. Individual *P. thomsoni* can be easily collected in native forest in AK but most populations are usually smaller than those found in urban habitats (pers. obs). This pattern has been previously observed in AB (Digweed 1998; Digweed *et al.*

1997) but it is not clear why it occurs. Anecdotal evidence suggests that, at least in AK, the pattern may be due to host resistance. In 2004 native birch forests outside of Anchorage that had never before showed evidence of significant leafminer damage were extensively defoliated (see Figure 5 in Snyder *et al.* 2007). This damage coincided with the hottest and driest summer on record in AK. It is possible that the drought conditions of 2004 may have reduced their ability of forest birch to resist *P thomsoni* defoliation (Dreistadt *et al.* 1990; Marino *et al.* 1993; McIntyre 2000; Zvereva and Kozlov 2006), thereby resulting in the significant damage. This hypothesis is supported by my observations of survivorship of *P. thomsoni* attacking urban birch in Anchorage during the same year. There, survivorship of eggs and first through fifth instar larvae was higher than in the other two years (Figure 3.7), which had more normal climate. If decreased tree resistance associated with drought was the cause of the extensive defoliation seen in 2004 it would support the hypothesis that trees in the urban forest are more severely damaged by *P. thomsoni* because they experience drought stress on a more consistent, yearly basis (Mattson and Haack 1987). Future studies may wish to examine the role of drought on birch leafminer performance.

Conclusion

Dealing with the threat of invasive species requires that we understand the organism at multiple scales. We must be able to devise tactics that minimize the impact of the species while at the same time understanding how population performance changes in response to environmental factors. It is important to understand and predict population changes through time, and then attempt to understand the processes that drive those patterns. Lastly it can be useful to know how an invasive species has changed, evolved and dispersed. In combination knowing these features for particular species will allow us to better predict how future introductions might occur, how organisms might behave and what actions could be taken to prevent further damage. Rather than see each introduction of an alien species as a single occurrence with unique effects, introductions should be treated as ‘natural experiments’ from which we learn about preventing future introductions and how to mitigate the effect when alien species become established. We can then begin to understand how adaptation and evolution act to shape the life history and ecology of species in new environments.

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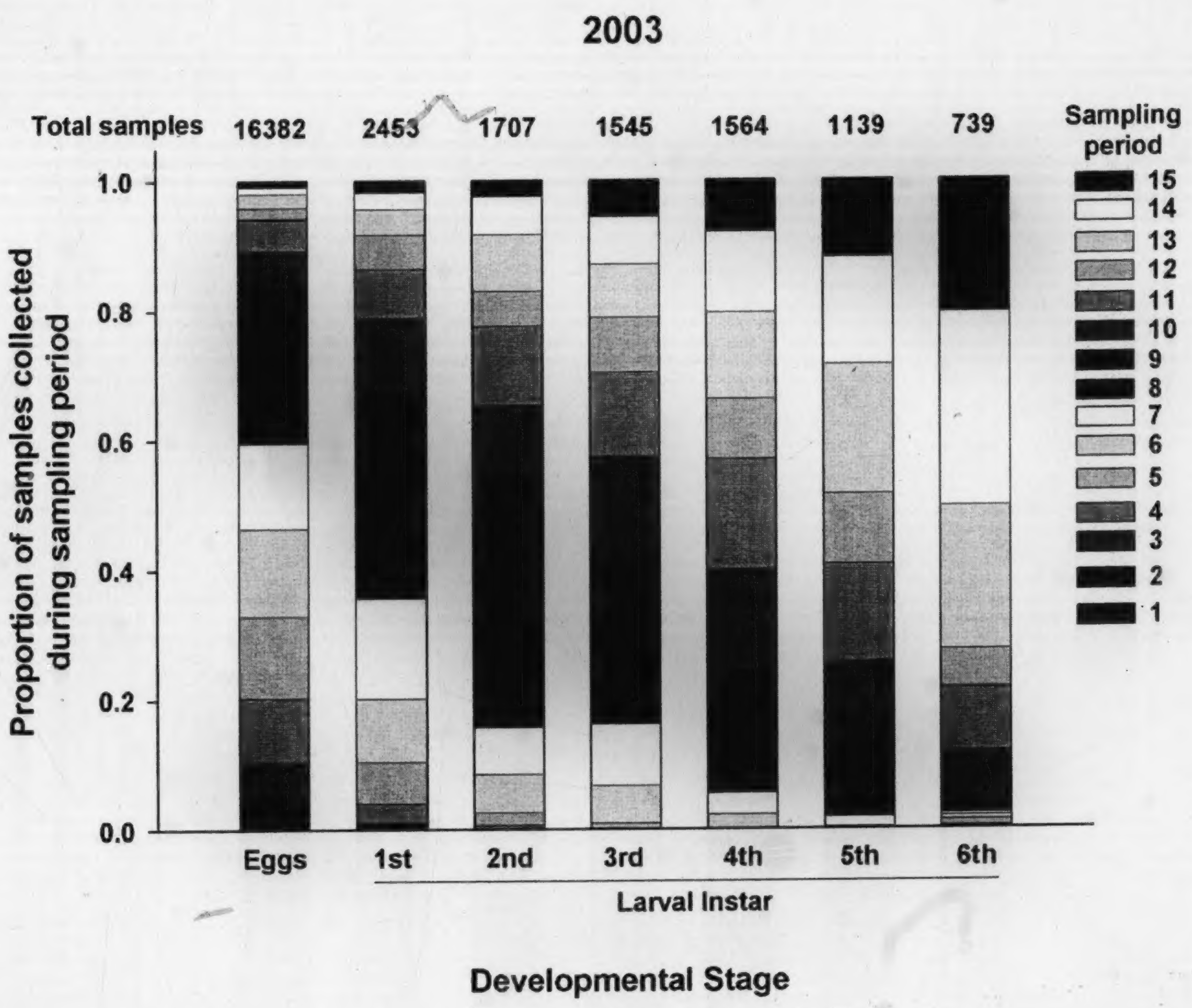
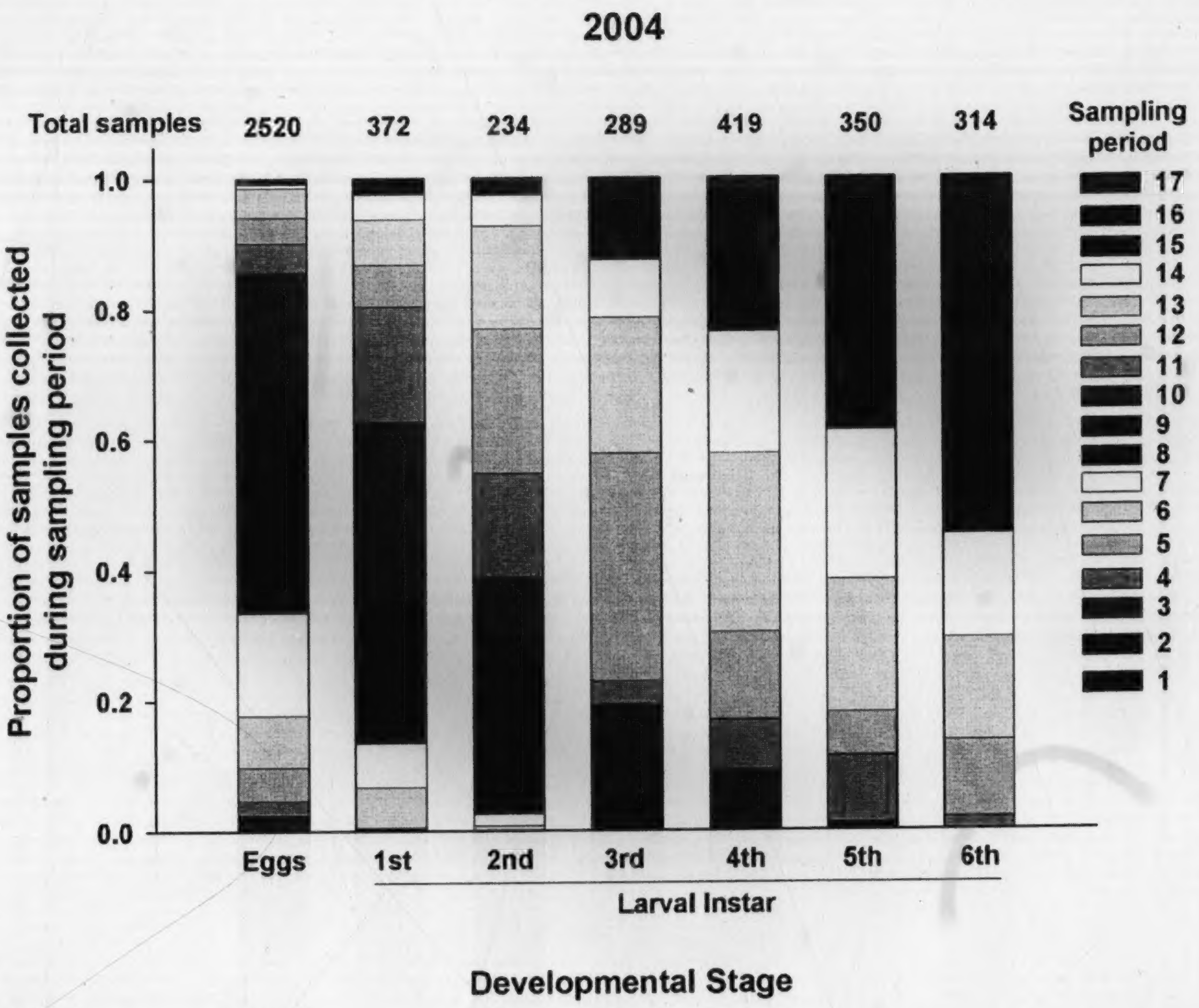
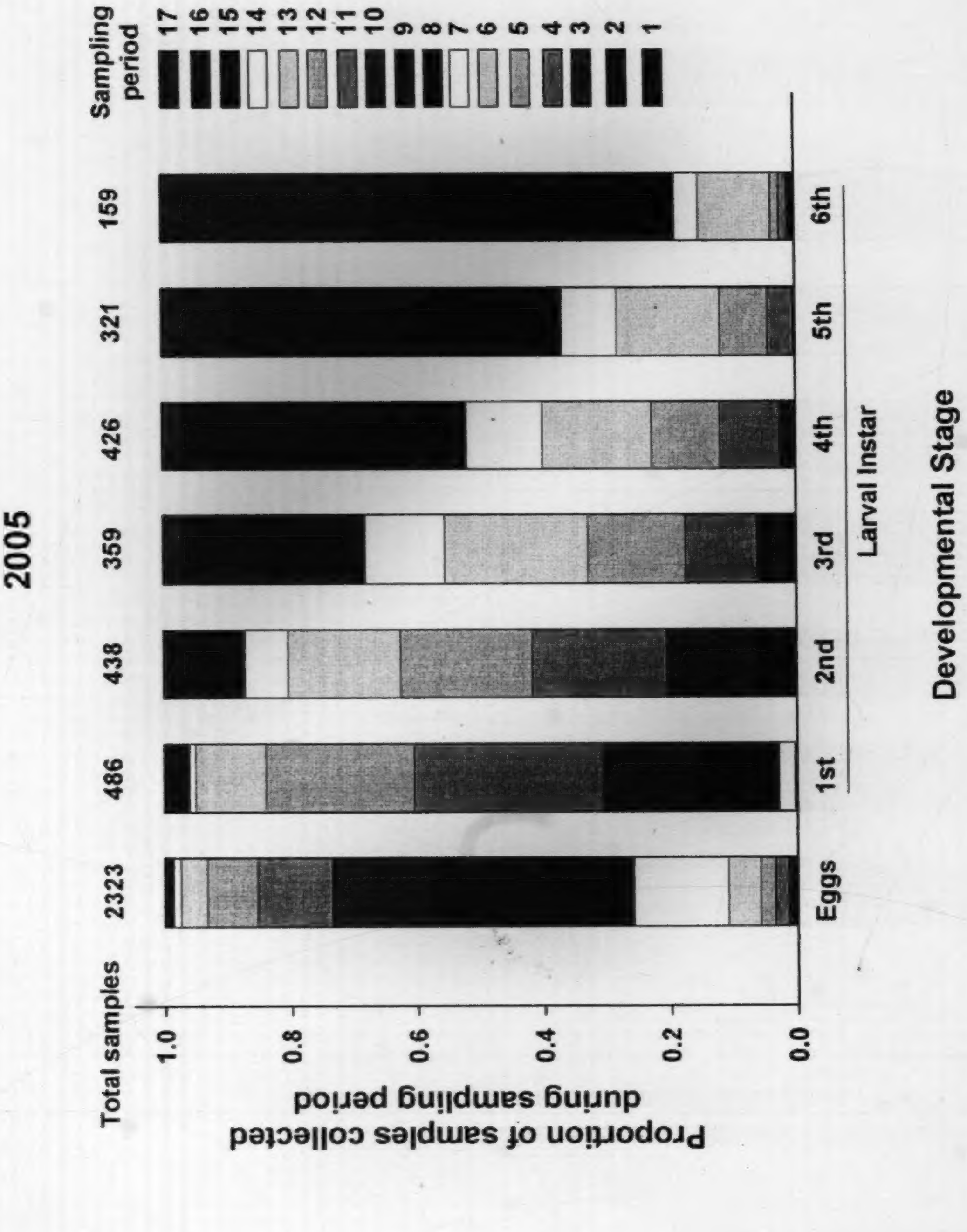
Appendix 1. Distribution of *Profenusa thomsoni* egg and larval samples across sample periods.

This appendix shows the distribution of *Profenusa thomsoni* egg and larval stages in samples taken from infested birch (*Betula*) trees in Anchorage, Alaska during 2003, 2004 and 2005. The egg stage and each larval instar are represented by a bar divided into segments representing the sample times. The segments are arranged in ascending chronological order and the height of each segment is scaled relative to the proportion of samples collected at each sample time. Sampling began in June of each year and sampling times were spaced 3-4 days apart in all years. The total number of individuals that were collected is given above each bar. Data for the 2003 figure were obtained from samples taken from each of three crown positions at eight sites, the 2004 and 2005 figures are constructed from data from three crown positions at two sites. Data from sites and crown positions were summed before plotting. See Chapter 3 for more details on the sampling method.


2003
Total samples
16382
2453
1707
1545
1564
1139
739
Proportion of samples collected during sampling period
1.0
0.8
0.6
0.4
0.2
0.0
Eggs
1st
2nd
3rd
4th
5th
6th
Larval Instar
Developmental Stage
Sampling period
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1


2004
Total samples
2520
372
234
289
419
350
314
Sampling period
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Proportion of samples collected during sampling period
1.0
0.8
0.6
0.4
0.2
0.0
Eggs
1st
2nd
3rd
4th
5th
6th
Larval Instar
Developmental Stage


2005
Total samples
2323
486
438
359
426
321
159
Proportion of samples collected during sampling period
1.0
0.8
0.6
0.4
0.2
0.0
Eggs
1st
2nd
3rd
4th
5th
6th
Larval Instar
Developmental Stage
Sampling period
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1

Appendix 2. KNM model outputs and simulation means

This appendix shows the stage specific survival rate (**stge sp. surv. rate**), stage duration (**stge dur.**) and number of individuals entering each developmental stage (**no. entering stge**) of *Profenusa thomsoni* egg and larval instars (**Stage**) at sample sites (**Site**) in Anchorage, Alaska during 2003, 2004 and 2005. The three parameters were derived for each of three crown heights (**Position**) at all sample sites. These parameters were derived using the Kiritani-Nakasuji-Manly model implemented in the P1F software. The values for each parameter were determined from observed stage frequencies; Mean values are determined from 100 simulated data sets with values drawn from Poisson distributions. Estimate values in bold are more than two standard errors different from the simulated mean and indicative of a poor fit of the model to the data. Estimate root mean square (**RMS**) is an indication of overall predictive fit of the KNM model parameters from the observed data to simulated data. If the RMS score for the observed values (**data**) is outside the distribution of RMS scores from 100 simulated sets (**range**) the parameters generated by the model do not predict the data. Development time is measured in degree days above a 5 C threshold for each stage; Unit time survival (**Unit time surv**.) is an internal model variable used to calculate the three parameters and is the stage-independent survival rate of larvae per degree day.

Terms in bold correspond to column headings in the following tables

Site	Position	Stage	Stge sp. surv. rate		Stge dur. (deg. days)		No. entering stge		Unit time surv.	RMS	
			Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE		data	range
Balto Seppala Park - 2003											
	Lower	Egg	0.477	0.477 ± 0.002	50.55	50.84 ± 0.62	1245	1248 ± 14	0.99	14.7	12.8 – 15.6
		L1	0.817	0.814 ± 0.003	**13.85**	14.14 ± 0.29	594	595 ± 7			
		L2	0.745	0.746 ± 0.004	20.13	20.13 ± 0.42	485	484 ± 6			
		L3	0.638	0.640 ± 0.004	30.68	30.66 ± 0.61	361	361 ± 5			
		L4	0.418	0.416 ± 0.006	59.65	60.31 ± 0.98	230	231 ± 4			
		L5	0.333	0.342 ± 0.009	75.07	73.75 ± 1.39	96	96 ± 2			
		L6	-	-	-	-	32	33 ± 1			
	Middle	Egg	0.420	0.421 ± 0.003	59.47	59.37 ± 1.00	746	753 ± 12	0.99	7.8	6.8 – 10.1
		L1	0.729	0.729 ± 0.004	21.73	21.66 ± 0.51	313	317 ± 6			
		L2	0.752	0.749 ± 0.005	19.57	19.82 ± 0.50	228	231 ± 4			
		L3	0.634	0.630 ± 0.007	31.23	31.78 ± 0.84	171	173 ± 4			
		L4	0.442	0.445 ± 0.010	56.08	55.66 ± 1.46	109	109 ± 2			
		L5	0.491	0.499 ± 0.014	48.82	48.01 ± 1.74	48	48 ± 1			
		L6	-	-	-	-	24	24 ± 1			
	Upper	Egg	0.518	0.517 ± 0.004	47.32	47.53 ± 0.94	435	438 ± 8	0.99	7.7	6.5 – 9.0
		L1	0.761	0.758 ± 0.005	19.63	19.98 ± 0.53	225	226 ± 5			
		L2	0.873	0.873 ± 0.005	9.73	9.85 ± 0.44	171	172 ± 4			
		L3	0.748	0.743 ± 0.007	20.84	21.34 ± 0.64	149	150 ± 3			
		L4	**0.709**	0.701 ± 0.008	**24.76**	25.60 ± 0.78	112	111 ± 2			
		L5	0.323	0.327 ± 0.010	81.36	80.69 ± 1.65	79	78 ± 2			
		L6	-	-	-	-	26	25 ± 1			
Jewel Lake 2003											
	Lower	Egg	0.371	0.371 ± 0.002	55.23	55.83 ± 0.80	1849	1841 ± 26	0.98	14.1	12.7 – 17.1
		L1	**0.720**	0.714 ± 0.003	18.91	18.97 ± 0.34	685	682 ± 10			
		L2	0.754	0.755 ± 0.004	15.71	15.79 ± 0.34	488	487 ± 8			
		L3	0.672	0.670 ± 0.005	22.14	22.50 ± 0.47	367	368 ± 6			
		L4	0.503	0.505 ± 0.006	38.25	38.41 ± 0.75	247	246 ± 4			
		L5	0.301	0.302 ± 0.007	66.73	67.74 ± 1.55	124	12 ± 25			
		L6	-	-	-	-	38	38 ± 1			

Site	Position	Stage	Stge sp. surv. rate		Stge dur. (deg. days)		No. entering stge		Unit time surv.	RMS	
			Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE		data	range
Jewel Lake 2003											
	Middle	Egg	0.381	0.382 ± 0.003	68.24	67.99 ± 0.93	**875**	886 ± 11	0.99	10.0	8.7 – 12.1
		L1	0.783	0.786 ± 0.004	17.29	16.99 ± 0.37	**333**	339 ± 5			
		L2	0.782	0.778 ± 0.004	17.42	17.72 ± 0.43	**261**	266 ± 4			
		L3	**0.727**	0.721 ± 0.006	22.59	23.09 ± 0.60	204	207 ± 4			
		L4	0.596	0.593 ± 0.006	36.72	36.90 ± 0.84	148	149 ± 3			
		L5	0.417	0.417 ± 0.009	61.92	61.79 ± 1.36	88	88 ± 2			
		L6	-	-	-	-	37	37 ± 1			
	Upper	Egg	0.312	0.313 ± 0.003	82.47	82.33 ± 1.45	762	768 ± 13	0.99	9.1	7.8 – 11.2
		L1	0.690	0.686 ± 0.006	26.31	26.68 ± 0.66	238	241 ± 5			
		L2	0.660	0.662 ± 0.006	29.39	29.21 ± 0.74	164	165 ± 3			
		L3	0.716	0.719 ± 0.008	23.66	23.65 ± 0.75	108	109 ± 2			
		L4	0.580	0.579 ± 0.008	38.62	38.88 ± 1.09	77	78 ± 2			
		L5	0.486	0.490 ± 0.011	51.14	50.00 ± 1.54	45	45 ± 1			
		L6	-	-	-	-	22	22 ± 1			
Javier Vega Park 2003											
	Lower	Egg	0.340	0.339 ± 0.002	66.54	66.76 ± 1.02	1451	1455 ± 20	0.98	28.1	25.4 – 31.4
		L1	**0.754**	0.749 ± 0.003	**17.38**	17.84 ± 0.37	492	493 ± 8			
		L2	0.716	0.717 ± 0.004	20.60	20.55 ± 0.46	371	369 ± 6			
		L3	0.597	0.595 ± 0.005	31.83	32.07 ± 0.63	266	265 ± 5			
		L4	0.525	0.523 ± 0.007	39.79	40.00 ± 0.87	158	158 ± 3			
		L5	0.272	0.278 ± 0.008	80.10	79.42 ± 1.72	83	82 ± 2			
		L6	-	-	-	-	23	23 ± 1			
	Middle	Egg	0.294	0.294 ± 0.003	76.61	76.21 ± 1.34	940	948 ± 16	0.98	10.2	8.6 – 12.6
		L1	0.640	0.642 ± 0.005	27.89	27.64 ± 0.67	276	279 ± 6			
		L2	0.721	0.725 ± 0.006	20.46	20.06 ± 0.63	176	179 ± 4			
		L3	0.780	0.779 ± 0.006	15.55	15.58 ± 0.53	127	130 ± 3			
		L4	0.471	0.463 ± 0.009	47.14	48.14 ± 1.25	99	101 ± 2			
		L5	0.267	0.266 ± 0.010	82.59	83.28 ± 2.15	46	47 ± 1			
		L6	-	-	-	-	13	12 ± 1			

Site	Position	Stage	Stge sp. surv. rate		Stge dur. (deg. days)		No. entering stge		Unit time surv.	RMS	
			Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE		data	range
Javier Vega Park 2003											
	Upper	Egg	0.461	0.462 ± 0.003	53.87	53.76 ± 0.59	1030	1028 ± 9	0.99	11.9	9.9 – 15.0
		L1	0.722	0.722 ± 0.004	22.67	22.70 ± 0.38	475	475 ± 5			
		L2	0.633	0.631 ± 0.005	31.87	32.02 ± 0.57	343	343 ± 4			
		L3	0.597	0.593 ± 0.006	35.94	36.37 ± 0.76	217	216 ± 3			
		L4	0.541	0.543 ± 0.007	42.71	42.54 ± 0.92	129	128 ± 2			
		L5	0.415	0.421 ± 0.010	61.17	60.38 ± 1.44	70	70 ± 1			
		L6	-	-	-	-	29	29 ± 1			
Javier Vega Park - 2004											
	Lower	Egg	0.626	0.625 ± 0.004	57.56	57.92 ± 1.14	264	265 ± 4	0.99	6.3	5.3 – 7.6
		L1	0.809	0.811 ± 0.004	26.02	25.86 ± 0.73	165	166 ± 3			
		L2	0.817	0.820 ± 0.004	24.79	24.44 ± 0.76	134	134 ± 3			
		L3	**0.839**	0.833 ± 0.005	**21.56**	22.40 ± 0.73	109	110 ± 2			
		L4	0.704	0.702 ± 0.007	43.07	43.48 ± 1.18	91	92 ± 2			
		L5	0.535	0.543 ± 0.009	76.80	75.20 ± 1.65	64	64 ± 1			
		L6	-	-	-	-	35	35 ± 1			
	Middle	Egg	0.701	0.702 ± 0.005	**49.17**	52.23 ± 1.18	**148**	140 ± 3	0.99	3.8	2.9 – 4.8
		L1	**0.753**	0.761 ± 0.005	**39.28**	41.33 ± 1.24	**103**	98 ± 2			
		L2	**0.812**	0.820 ± 0.006	28.74	29.86 ± 1.15	**78**	74 ± 2			
		L3	**0.927**	0.932 ± 0.004	10.43	10.61 ± 0.70	**63**	61 ± 1			
		L4	**0.723**	0.737 ± 0.008	44.85	46.05 ± 1.59	**58**	57 ± 1			
		L5	**0.635**	0.654 ± 0.011	62.77	63.97 ± 1.95	42	42 ± 1			
		L6	-	-	-	-	27	27 ± 1			
	Upper	Egg	0.492	0.494 ± 0.005	119.9	119.04 ± 2.16	154	155 ± 3	0.99	2.6	2.2 – 4.3
		L1	0.755	0.751 ± 0.008	47.50	48.47 ± 1.75	75	77 ± 2			
		L2	0.881	0.881 ± 0.006	21.40	21.45 ± 1.10	57	58 ± 1			
		L3	0.878	0.881 ± 0.007	21.92	21.51 ± 1.31	50	51 ± 1			
		L4	0.775	0.772 ± 0.008	43.10	43.71 ± 1.75	44	45 ± 1			
		L5	0.810	0.811 ± 0.009	35.64	35.27 ± 1.63	**31**	34 ± 1			
		L6	-	-	-	-	28	28 ± 1			

Site	Position	Stage	Stge sp. surv. rate		Stge dur. (deg. days)		No. entering stge		Unit time surv.	RMS	
			Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE		data	range
Javier Vega Park 2005											
	Lower	Egg	**0.377**	0.380 ± 0.003	**36.52**	35.73 ± 0.64	**2054**	2108 ± 39	0.97	14.1	13.0 – 16.0
		L1	0.670	0.669 ± 0.004	14.98	14.84 ± 0.33	**774**	800 ± 16			
		L2	0.608	0.609 ± 0.005	18.64	18.31 ± 0.42	**518**	535 ± 11			
		L3	**0.593**	0.586 ± 0.007	19.55	19.76 ± 0.53	**315**	326 ± 7			
		L4	0.315	0.313 ± 0.007	43.27	42.97 ± 0.87	187	191 ± 5			
		L5	0.063	0.062 ± 0.006	103.72	105.34 ± 2.30	58	59 ± 2			
		L6	-	-	-	-	4	4 ± 1			
	Middle	Egg	0.482	0.485 ± 0.004	30.33	30.11 ± 0.80	745	757 ± 17	0.98	7.5	5.9 – 8.7
		L1	0.778	0.780 ± 0.005	10.42	10.32 ± 0.30	359	367 ± 10			
		L2	0.729	0.733 ± 0.006	13.15	12.90 ± 0.41	279	286 ± 8			
		L3	0.634	0.636 ± 0.006	18.91	18.78 ± 0.50	**203**	210 ± 6			
		L4	0.461	0.464 ± 0.009	32.16	31.94 ± 0.93	**129**	133 ± 4			
		L5	0.302	0.302 ± 0.013	49.75	50.23 ± 1.67	**59**	62 ± 2			
		L6	-	-	-	-	18	19 ± 1			
	Upper	Egg	0.552	0.550 ± 0.005	24.60	24.51 ± 0.64	520	530 ± 13	0.98	5.7	4.9 – 7.3
		L1	0.785	0.789 ± 0.006	10.04	9.74 ± 0.37	287	292 ± 7			
		L2	0.678	0.677 ± 0.008	16.10	16.02 ± 0.59	225	230 ± 6			
		L3	0.638	0.640 ± 0.009	18.62	18.33 ± 0.66	152	156 ± 5			
		L4	0.498	0.494 ± 0.011	28.92	29.07 ± 1.14	97	99 ± 3			
		L5	0.250	0.240 ± 0.013	57.37	59.49 ± 2.30	48	49 ± 2			
		L6	-	-	-	-	12	12 ± 1			
Loch Lomand Road -2003											
	Lower	Egg	0.281	0.282 ± 0.004	65.31	64.48 ± 1.60	519	532 ± 13	0.98	4.9	4.1 – 6.1
		L1	0.666	0.666 ± 0.007	20.97	20.78 ± 0.68	146	150 ± 4			
		L2	**0.684**	0.672 ± 0.009	19.59	20.32 ± 0.80	97	100 ± 3			
		L3	0.649	0.647 ± 0.011	22.28	22.29 ± 0.92	66	67 ± 2			
		L4	0.444	0.453 ± 0.013	41.80	40.72 ± 1.55	43	43 ± 1			
		L5	0.206	0.200 ± 0.016	**81.34**	85.25 ± 3.82	19	20 ± 1			
		L6	-	-	-	-	4	4 ± 1			

Site	Position	Stage	Stge sp. surv. rate		Stge dur. (deg. days)		No. entering stge		Unit time surv.	RMS	
			Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE		data	range
Loch Lomond Road -2003											
	Middle	Egg	0.216	0.214 ± 0.004	**65.94**	58.02 ± 4.58	**411**	2699 ± 1190	0.98	2.7	2.2 – 108.3
		L1	0.536	0.546 ± 0.011	**26.80**	22.64 ± 1.96	**89**	572 ± 254			
		L2	0.655	0.660 ± 0.014	**18.19**	15.42 ± 1.43	**47**	300 ± 134			
		L3	0.397	0.406 ± 0.016	**39.74**	34.25 ± 3.06	**31**	188 ± 83			
		L4	0.432	0.431 ± 0.025	36.15	32.87 ± 3.46	**12**	73 ± 32			
		L5	0.157	0.153 ± 0.021	79.72	-	5	30 ± 13			
		L6	-	-	-	-	1	1 ± 1			
	Upper	Egg	0.361	0.363 ± 0.005	51.00	51.34 ± 1.39	421	424 ± 11	0.98	4.4	3.7 – 6.2
		L1	0.869	0.871 ± 0.006	7.03	7.00 ± 0.36	152	154 ± 5			
		L2	0.648	0.654 ± 0.008	21.72	21.53 ± 0.76	132	134 ± 4			
		L3	0.773	0.768 ± 0.009	12.89	13.38 ± 0.64	85	88 ± 3			
		L4	0.542	0.541 ± 0.011	30.66	31.11 ± 1.09	66	67 ± 2			
		L5	0.455	0.456 ± 0.014	39.49	39.99 ± 1.54	36	36 ± 1			
		L6	-	-	-	-	16	16 ± 1			
Ocean View Park - 2003											
	Lower	Egg	0.429	0.429 ± 0.002	38.90	39.25 ± 0.96	1961	1973 ± 46	0.98	13.5	11.3 – 15.6
		L1	0.748	0.749 ± 0.004	13.33	13.38 ± 0.38	842	847 ± 21			
		L2	0.704	0.704 ± 0.004	16.18	16.28 ± 0.46	630	635 ± 16			
		L3	0.638	0.640 ± 0.005	20.68	20.68 ± 0.60	443	447 ± 12			
		L4	0.414	0.411 ± 0.006	40.86	41.26 ± 1.10	282	286 ± 8			
		L5	0.211	0.213 ± 0.007	71.56	72.10 ± 1.93	116	118 ± 3			
		L6	-	-	-	-	25	25 ± 1			
	Middle	Egg	0.398	0.397 ± 0.003	41.17	41.02 ± 1.18	1117	1149 ± 36	0.98	7.7	6.4 – 10.7
		L1	0.694	0.693 ± 0.005	16.31	16.25 ± 0.52	444	456 ± 15			
		L2	0.721	0.721 ± 0.005	14.64	14.49 ± 0.50	308	316 ± 10			
		L3	0.649	0.646 ± 0.007	19.29	19.36 ± 0.67	222	228 ± 7			
		L4	0.408	0.406 ± 0.009	40.05	40.19 ± 1.46	144	147 ± 5			
		L5	0.293	0.291 ± 0.013	54.91	55.77 ± 2.45	58	60 ± 2			
		L6	-	-	-	-	17	17 ± 1			

Site	Position	Stage	Stge sp. surv. rate		Stge dur. (deg. days)		No. entering stge		Unit time surv.	RMS	
			Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE		data	range
Ocean View Park - 2003											
	Upper	Egg	0.343	0.344 ± 0.003	29.84	30.60 ± 1.85	1819	2159 ± 352	0.96	8.3	6.7 – 56.7
		L1	0.603	0.606 ± 0.006	14.13	14.33 ± 0.84	624	745 ± 123			
		L2	0.521	0.520 ± 0.007	18.22	18.79 ± 1.17	376	453 ± 79			
		L3	0.510	0.517 ± 0.009	17.79	18.95 ± 1.21	195	236 ± 42			
		L4	0.408	0.406 ± 0.013	24.99	26.18 ± 1.86	**99**	122 ± 22			
		L5	**0.438**	0.459 ± 0.018	23.01	22.67 ± 1.76	40	49 ± 9			
		L6	-	-	-	-	**18**	22 ± 4			
Russian Jack Park - 2003											
	Lower	Egg	0.069	0.070 ± 0.002	**76.56**	67.21 ± 5.82	**1485**	6882 ± 2774	0.97	10.5	8.0 – 333.2
		L1	0.186	0.180 ± 0.013	**48.32**	43.72 ± 4.16	**102**	446 ± 178			
		L2	0.282	0.316 ± 0.044	36.29	-	**19**	62 ± 23			
		L3	-	-	-	-	5	6 ± 1			
	Middle	Egg	0.074	0.074 ± 0.002	**83.29**	68.90 ± 6.43	**1175**	7695 ± 2789	0.97	7.0	4.9 – 285.8
		L1	**0.211**	0.196 ± 0.012	**51.34**	43.22 ± 4.36	**87**	542 ± 197			
		L2	0.597	0.580 ± 0.033	**16.54**	14.06 ± 1.87	**17**	89 ± 32			
		L3	0.325	0.309 ± 0.046	36.07	-	**10**	45 ± 18			
		L4	-	-	-	-	3	3 ± 1			
	Upper	Egg	0.155	0.155 ± 0.002	55.67	55.33 ± 1.30	**2153**	2225 ± 61	0.97	12.4	9.7 – 16.6
		L1	0.475	0.475 ± 0.009	22.55	22.15 ± 0.64	**332**	346 ± 10			
		L2	0.656	0.657 ± 0.011	12.79	12.56 ± 0.52	**158**	164 ± 5			
		L3	0.715	0.713 ± 0.011	10.14	10.08 ± 0.46	**103**	107 ± 3			
		L4	0.555	0.569 ± 0.016	**17.83**	16.85 ± 0.76	74	76 ± 2			
		L5	0.243	0.237 ± 0.018	42.89	44.09 ± 1.77	**41**	43 ± 2			
		L6	-	-	-	-	10	10 ± 1			

Site	Position	Stage	Stge sp. surv. rate		Stge dur. (deg. days)		No. entering stge		Unit time surv.	RMS	
			Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE		data	range
Russian Jack Park - 2004											
	Lower	Egg	0.331	0.332 ± 0.003	90.55	89.98 ± 1.82	**862**	880 ± 18	0.99	12.9	10.4 – 15.9
		L1	0.775	0.773 ± 0.004	20.85	20.85 ± 0.55	**285**	292 ± 6			
		L2	0.852	0.853 ± 0.004	13.09	12.85 ± 0.44	220	225 ± 5			
		L3	0.739	0.738 ± 0.005	24.71	24.65 ± 0.77	188	192 ± 4			
		L4	0.514	0.514 ± 0.007	54.40	53.91 ± 1.31	139	142 ± 3			
		L5	0.284	0.280 ± 0.010	103.01	103.42 ± 2.25	71	73 ± 2			
		L6	-	-	-	-	20	20 ± 1			
	Middle	Egg	0.443	0.446 ± 0.003	63.91	63.65 ± 1.20	755	755 ± 14	0.99	10.6	9.3 – 12.8
		L1	0.836	0.836 ± 0.004	13.99	14.13 ± 0.39	334	337 ± 7			
		L2	0.844	0.842 ± 0.004	13.28	13.56 ± 0.46	279	281 ± 6			
		L3	0.703	0.701 ± 0.006	27.48	27.95 ± 0.71	235	237 ± 5			
		L4	0.542	0.541 ± 0.007	47.88	48.32 ± 1.08	166	166 ± 3			
		L5	0.328	0.330 ± 0.010	87.12	87.36 ± 1.72	89	90 ± 2			
		L6	-	-	-	-	30	30 ± 1			
	Upper	Egg	0.491	0.491 ± 0.004	70.39	70.90 ± 1.24	637	639 ± 9	0.99	8.6	7.3 – 10.5
		L1	0.875	0.876 ± 0.003	13.18	13.15 ± 0.42	313	314 ± 5			
		L2	0.881	0.884 ± 0.004	12.56	12.25 ± 0.42	274	275 ± 5			
		L3	0.739	0.740 ± 0.005	29.93	29.97 ± 0.72	241	243 ± 4			
		L4	0.551	0.550 ± 0.007	59.12	59.52 ± 1.29	178	180 ± 3			
		L5	0.443	0.446 ± 0.008	80.72	80.40 ± 1.49	98	99 ± 2			
		L6	-	-	-	-	44	44 ± 1			
Russian Jack Park - 2005											
	Lower	Egg	0.467	0.465 ± 0.004	40.82	41.79 ± 0.98	732	723 ± 18	0.98	7.8	6.7 – 9.5
		L1	0.760	0.757 ± 0.005	**14.62**	15.16 ± 0.46	**341**	356 ± 8			
		L2	0.719	0.720 ± 0.006	17.55	17.92 ± 0.59	259	254 ± 6			
		L3	0.693	0.693 ± 0.007	19.48	20.02 ± 0.69	186	183 ± 5			
		L4	0.501	0.501 ± 0.009	36.72	37.82 ± 1.22	129	127 ± 3			
		L5	0.399	0.399 ± 0.014	48.91	50.50 ± 1.80	64	64 ± 2			
		L6	-	-	-	-	25	25 ± 1			

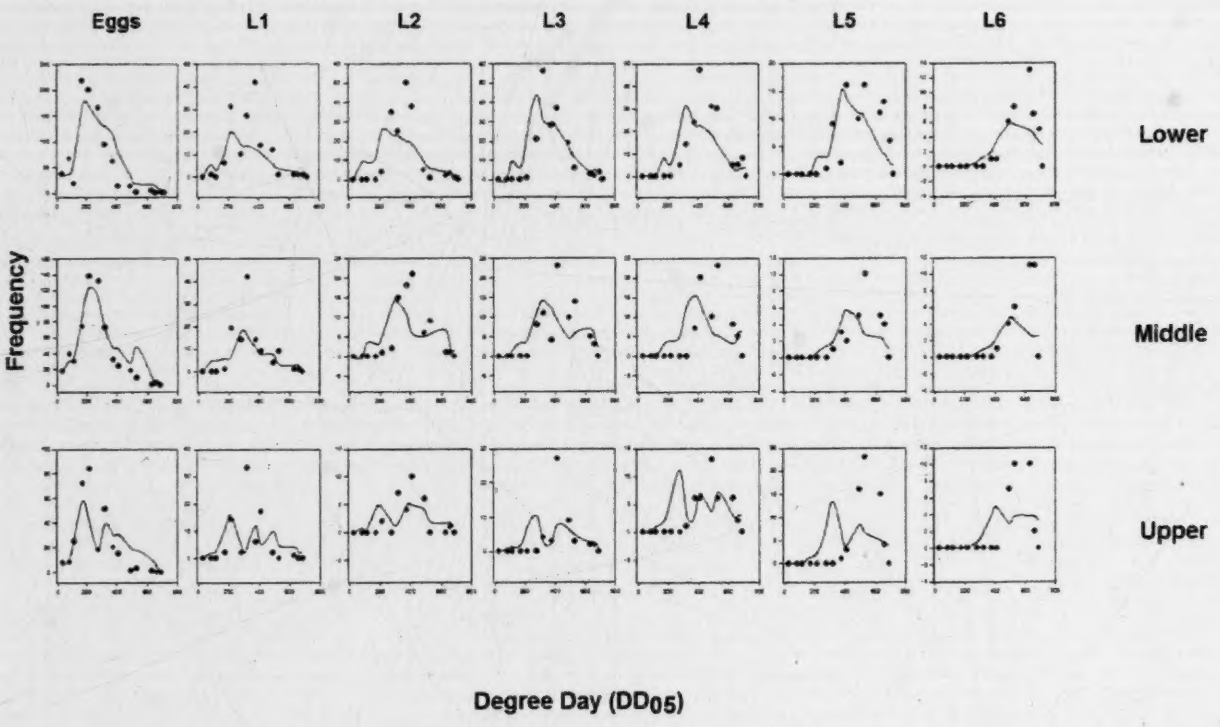
Site	Position	Stage	Stge sp. surv. rate		Stge dur. (deg. days)		No. entering stge		Unit time surv.	RMS	
			Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE		data	range
Russian Jack Park - 2005											
	Middle	Egg	0.557	0.559 ± 0.004	**36.27**	35.49 ± 0.58	**547**	562 ± 9	0.98	5.9	5.4 – 7.5
		L1	0.781	0.782 ± 0.004	15.34	15.00 ± 0.37	**305**	314 ± 5			
		L2	0.745	0.744 ± 0.006	18.31	18.08 ± 0.56	**238**	246 ± 4			
		L3	0.710	0.709 ± 0.006	21.30	21.05 ± 0.56	**177**	183 ± 4			
		L4	0.570	0.577 ± 0.010	**34.88**	33.70 ± 0.99	**125**	130 ± 3			
		L5	0.237	0.231 ± 0.010	89.28	90.21 ± 2.08	**71**	75 ± 2			
		L6	-	-	-	-	17	17 ± 1			
	Upper	Egg	0.431	0.429 ± 0.004	61.42	61.97 ± 1.26	479	479 ± 8	0.99	7.1	6.2 – 8.7
		L1	0.821	0.817 ± 0.005	14.33	14.77 ± 0.48	206	206 ± 5			
		L2	0.807	0.806 ± 0.005	15.60	15.79 ± 0.55	169	168 ± 4			
		L3	0.794	0.799 ± 0.007	16.86	16.47 ± 0.63	136	136 ± 3			
		L4	0.511	0.515 ± 0.010	48.90	48.69 ± 1.38	108	108 ± 3			
		L5	0.286	0.287 ± 0.010	91.12	91.70 ± 2.08	55	56 ± 2			
		L6	-	-	-	-	16	16 ± 1			
Westchester Lagoon - 2003											
	Lower	Egg	0.514	0.512 ± 0.005	65.74	65.96 ± 1.40	**280**	241 ± 5	0.99	7.8	7.0 – 9.5
		L1	**0.860**	0.864 ± 0.004	14.82	14.35 ± 0.48	143	144 ± 3			
		L2	0.905	0.904 ± 0.003	9.80	9.91 ± 0.39	123	124 ± 3			
		L3	0.865	0.866 ± 0.005	14.25	14.17 ± 0.62	111	112 ± 2			
		L4	0.684	0.687 ± 0.007	37.24	36.87 ± 0.95	96	97 ± 2			
		L5	0.600	0.598 ± 0.010	50.06	50.61 ± 1.46	66	67 ± 1			
		L6	-	-	-	-	40	40 ± 1			
	Middle	Egg	0.452	0.450 ± 0.004	48.68	49.61 ± 1.45	404	408 ± 16	0.98	6.1	4.7 – 7.7
		L1	0.749	0.747 ± 0.006	17.75	18.13 ± 0.68	182	184 ± 8			
		L2	0.753	0.757 ± 0.007	17.40	17.26 ± 0.63	136	137 ± 6			
		L3	0.827	0.823 ± 0.008	11.67	12.07 ± 0.65	102	104 ± 4			
		L4	0.635	0.630 ± 0.011	27.84	28.85 ± 1.31	85	85 ± 3			
		L5	**0.307**	0.322 ± 0.013	72.29	70.85 ± 2.55	53	54 ± 2			
		L6	-	-	-	-	17	17 ± 1			

Site	Position	Stage	Stge sp. surv. rate		Stge dur. (deg. days)		No. entering stge		Unit time surv.	RMS	
			Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE	Est.	$\bar{X}$ ± 2SE		data	range
Westchester Lagoon - 2003											
	Upper	Egg	0.319	0.323 ± 0.004	60.18	59.16 ± 1.06	727	733 ± 12	0.98	8.4	6.8 – 10.2
		L1	0.627	0.629 ± 0.006	24.61	24.35 ± 0.62	232	237 ± 5			
		L2	0.697	0.691 ± 0.009	19.01	19.39 ± 0.70	**145**	149 ± 3			
		L3	**0.699**	0.678 ± 0.009	**18.85**	20.38 ± 0.73	101	103 ± 3			
		L4	0.519	0.506 ± 0.013	**34.59**	35.94 ± 1.32	71	70 ± 2			
		L5	**0.327**	0.344 ± 0.016	58.88	56.76 ± 2.22	36	35 ± 1			
		L6	-	-	-	-	12	12 ± 1			
Government Hill (Water Tower) - 2003											
	Lower	Egg	0.343	0.343 ± 0.003	85.41	85.13 ± 1.06	762	769 ± 10	0.99	6.3	5.6 – 8.0
		L1	0.760	0.764 ± 0.005	21.89	21.46 ± 0.51	261	264 ± 4			
		L2	0.866	0.864 ± 0.004	11.47	11.61 ± 0.35	199	201 ± 3			
		L3	0.715	0.715 ± 0.006	26.78	26.67 ± 0.67	172	174 ± 3			
		L4	0.621	0.618 ± 0.007	38.09	38.30 ± 0.89	123	124 ± 2			
		L5	0.479	0.483 ± 0.010	58.86	58.05 ± 1.39	76	77 ± 2			
		L6	-	-	-	-	37	37 ± 1			
	Middle	Egg	**0.334**	0.338 ± 0.003	52.36	52.32 ± 1.10	814	820 ± 18	0.98	5.1	4.4 – 6.7
		L1	0.709	0.710 ± 0.006	16.41	16.51 ± 0.50	271	277 ± 6			
		L2	0.767	0.766 ± 0.006	12.68	12.85 ± 0.44	192	196 ± 5			
		L3	0.803	0.803 ± 0.006	10.48	10.64 ± 0.44	147	150 ± 4			
		L4	0.565	0.567 ± 0.009	27.29	27.42 ± 0.86	118	121 ± 3			
		L5	0.401	0.400 ± 0.012	43.60	44.36 ± 1.29	67	68 ± 2			
		L6	-	-	-	-	**30**	27 ± 1			
	Upper	Egg	0.311	0.311 ± 0.003	54.68	53.59 ± 1.20	**1367**	1415 ± 35	0.98	5.6	5.0 – 8.0
		L1	0.716	0.719 ± 0.005	15.61	15.09 ± 0.45	**425**	440 ± 12			
		L2	**0.767**	0.761 ± 0.005	12.44	12.52 ± 0.41	**304**	316 ± 8			
		L3	0.647	0.646 ± 0.007	20.39	20.00 ± 0.60	**233**	241 ± 7			
		L4	0.611	0.610 ± 0.009	23.04	22.74 ± 0.82	**151**	155 ± 4			
		L5	0.196	0.193 ± 0.010	76.27	76.22 ± 2.23	92	95 ± 3			
		L6	-	-	-	-	18	18 ± 1			

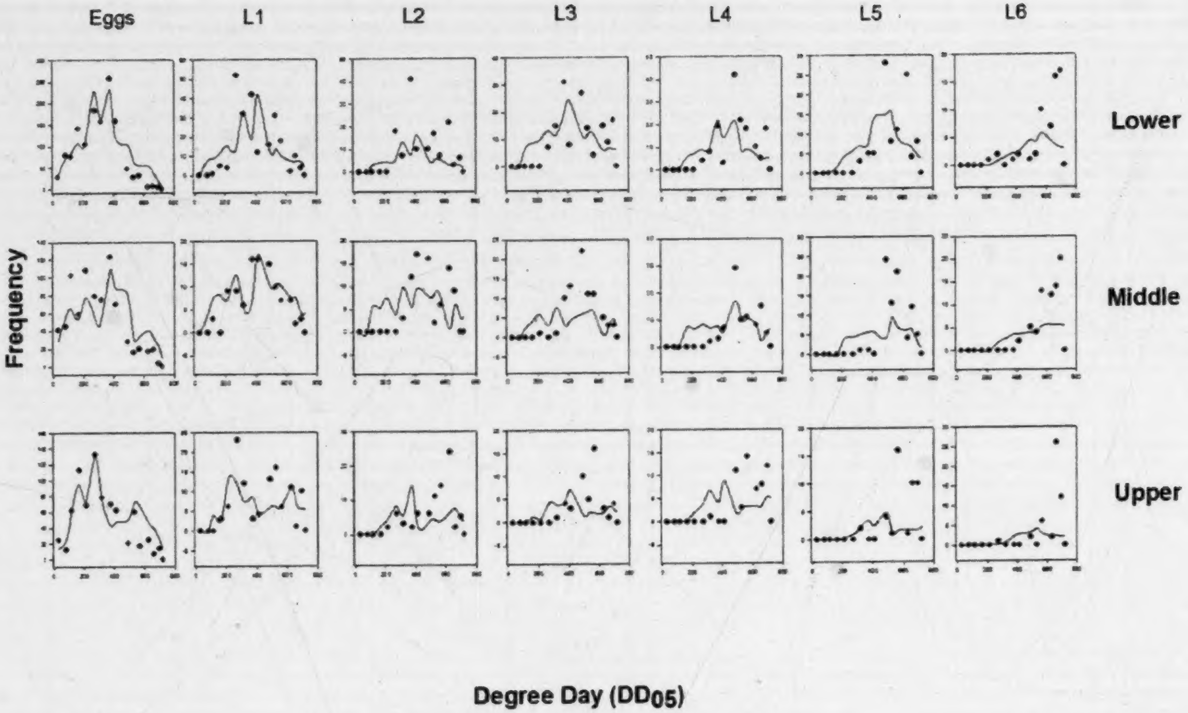
Appendix 3. Observed versus expected stage frequency plots

Graphical comparison of observed (dots) and predicted (lines) stage frequency distributions for egg and larval stages of *P. thomsoni* in Anchorage, Alaska. Predicted frequency distributions are determined by applying population parameters derived from the observed dataset to a simulated set having the same entry distribution. Site and year are given at the top of each figure.

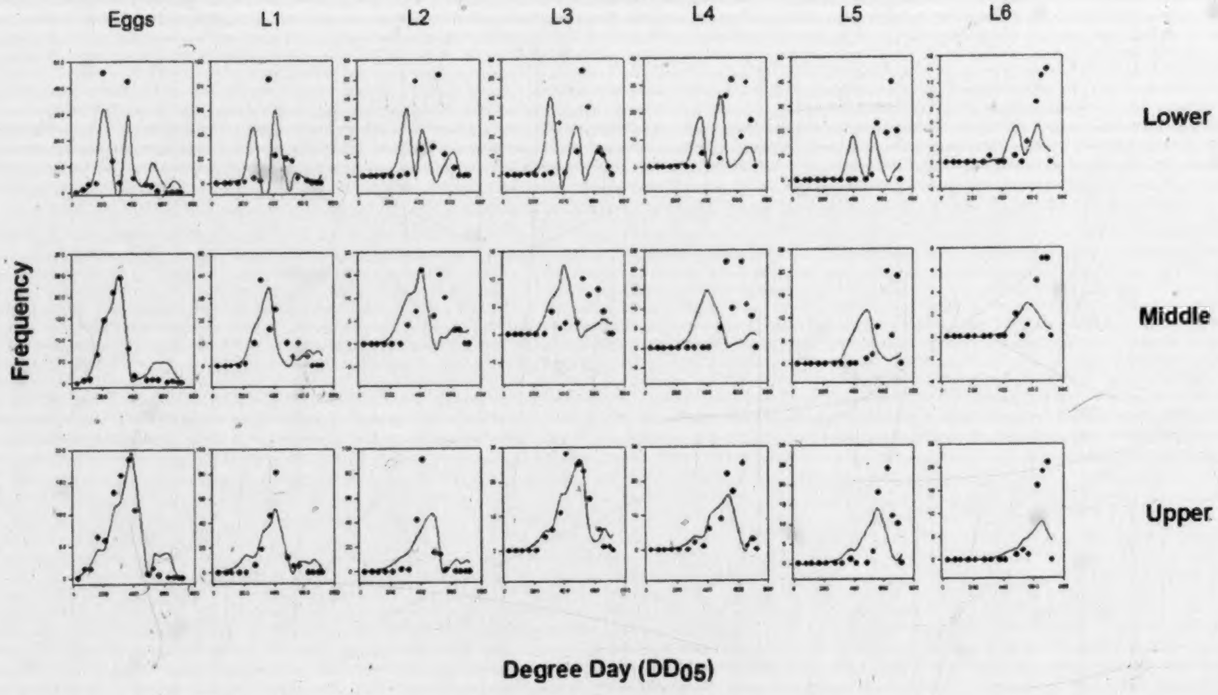
Balto Seppela Park 2003



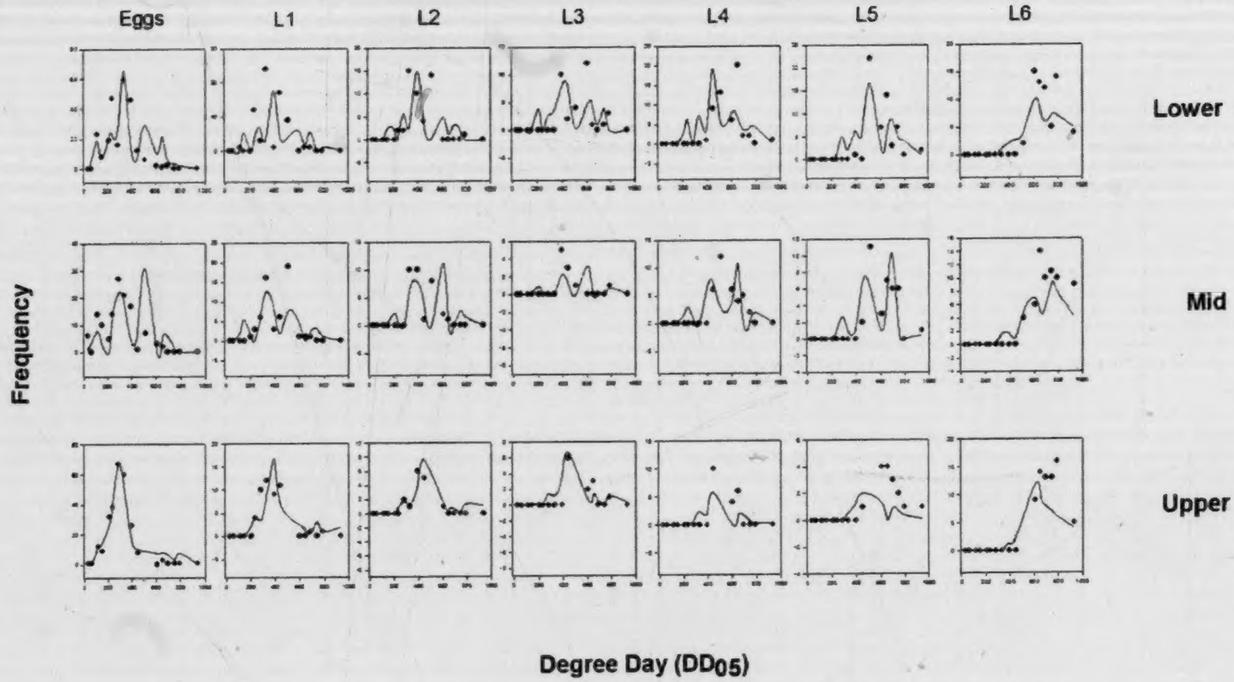
Jewel Lake Park 2003



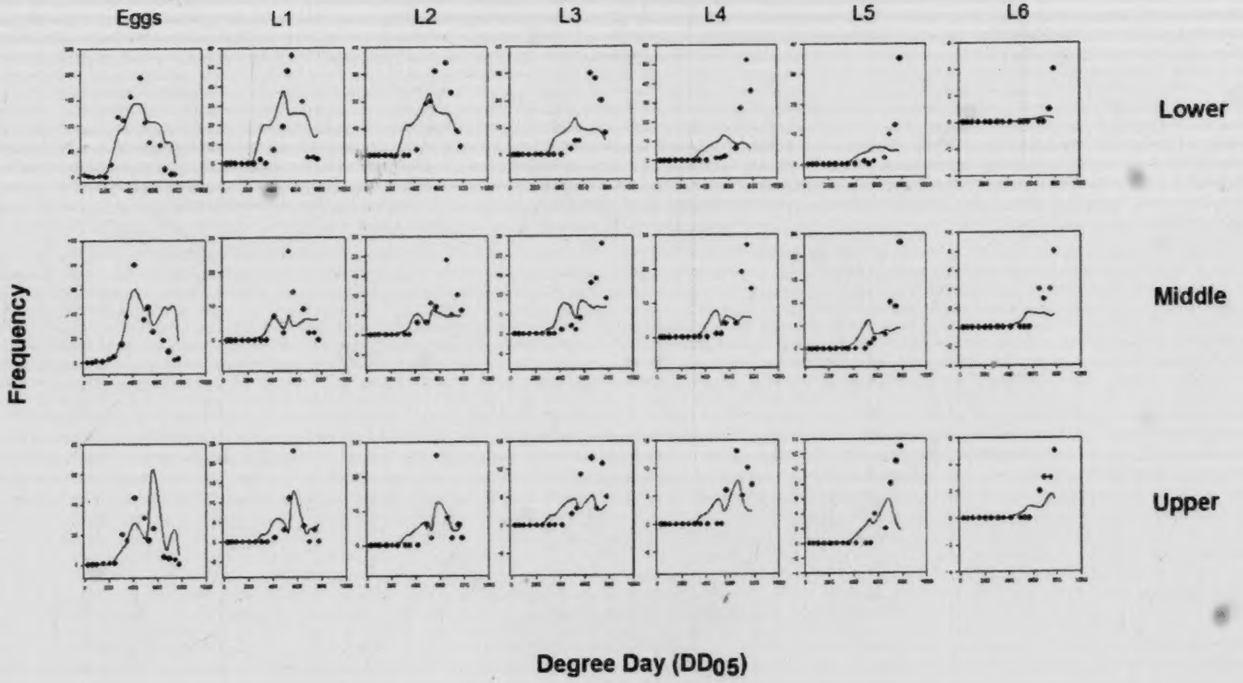
Javier de la Vega Park 2003



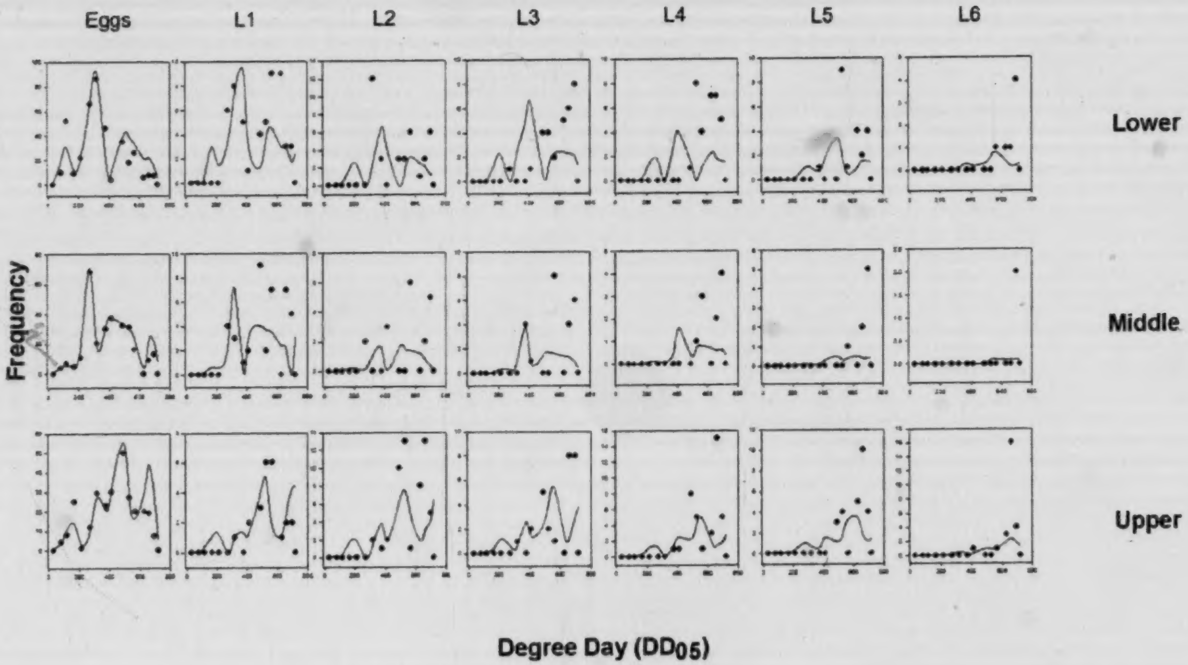
Javier de la Vega Park 2004



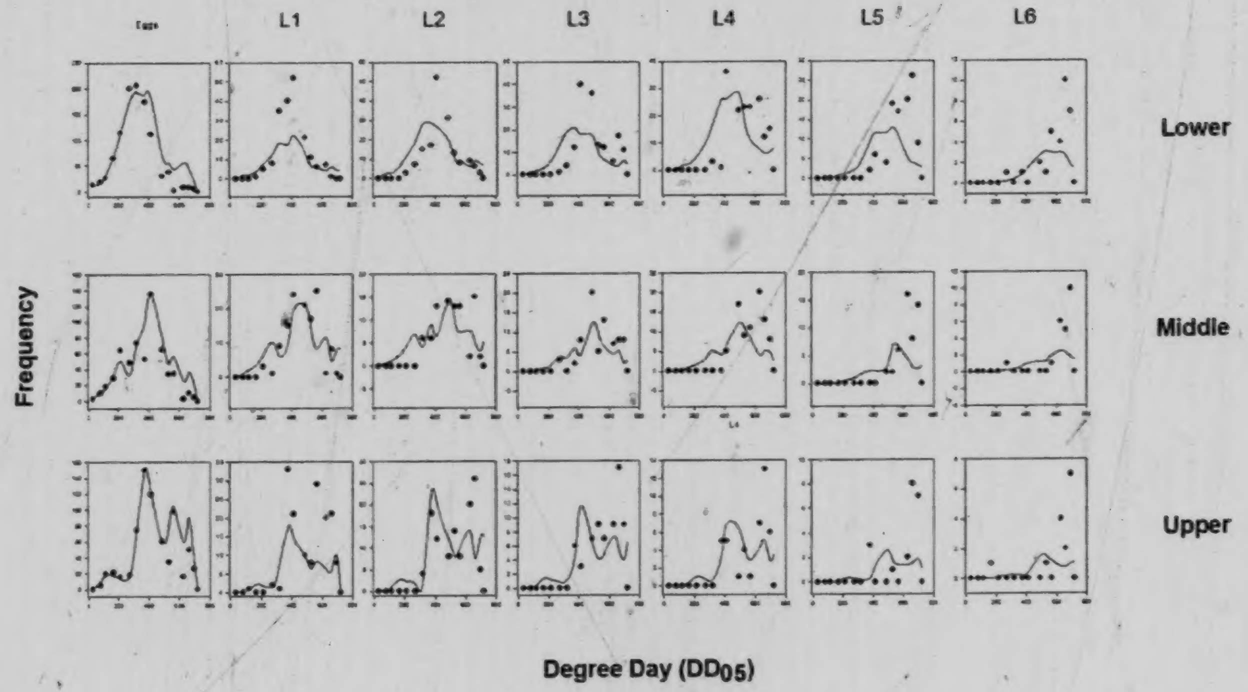
Javier de la Vega Park 2005



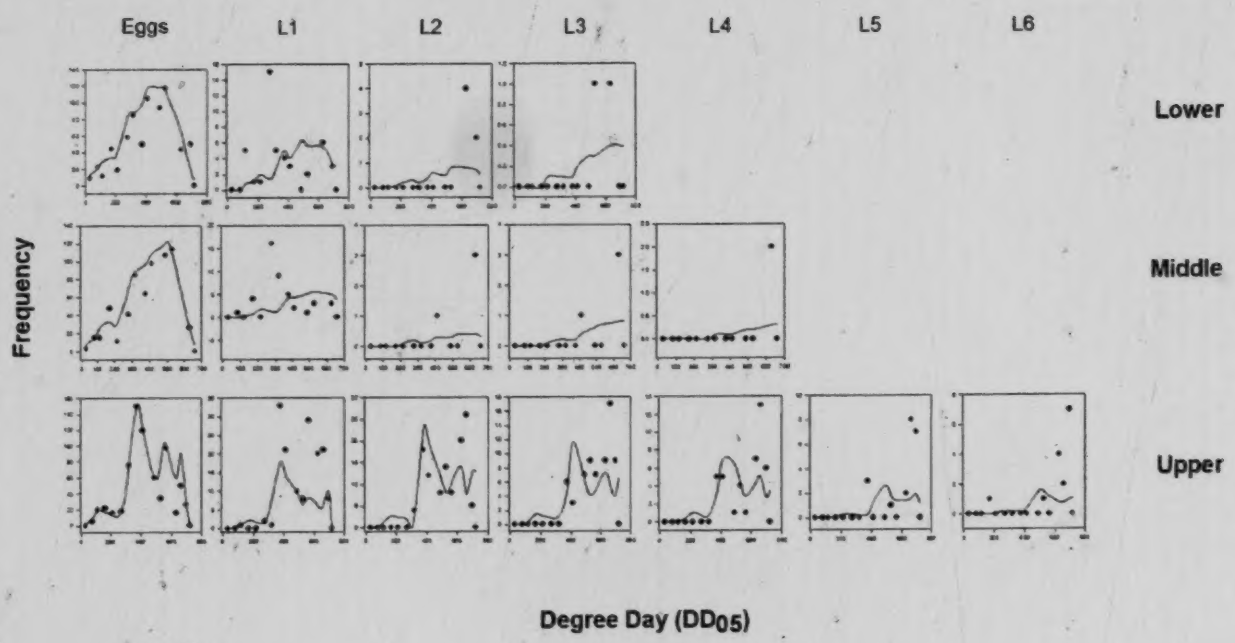
Loch Lomand Road 2003



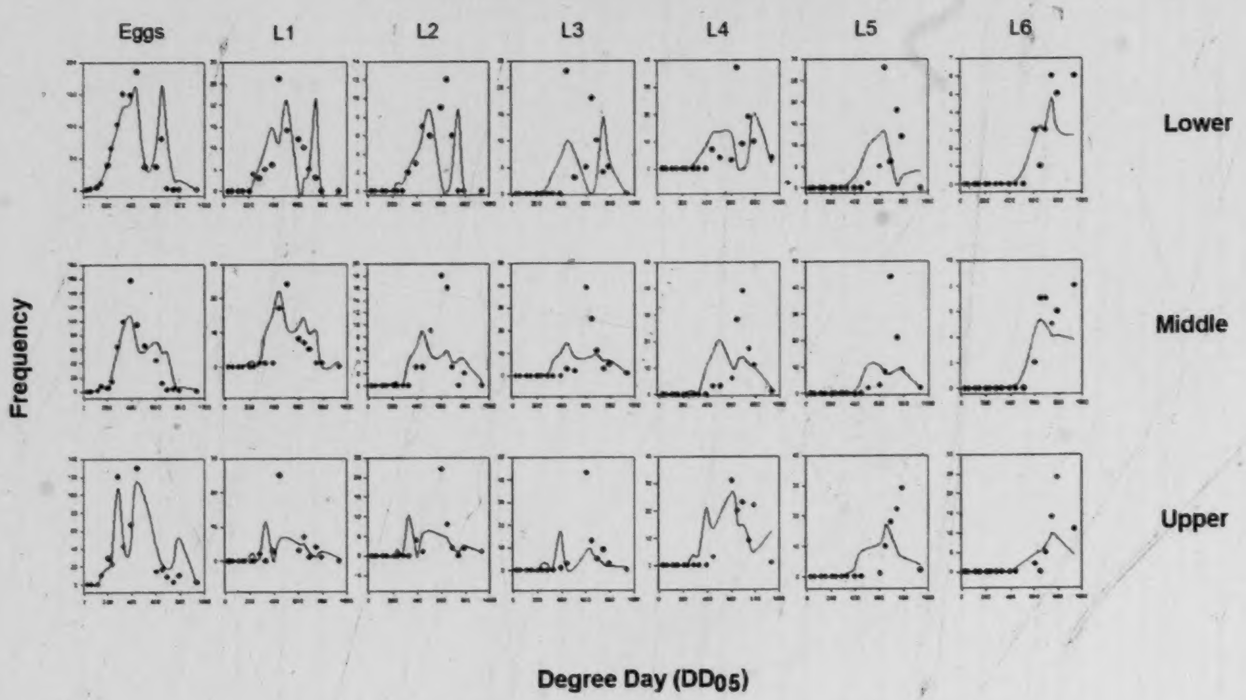
Ocean View Park 2003



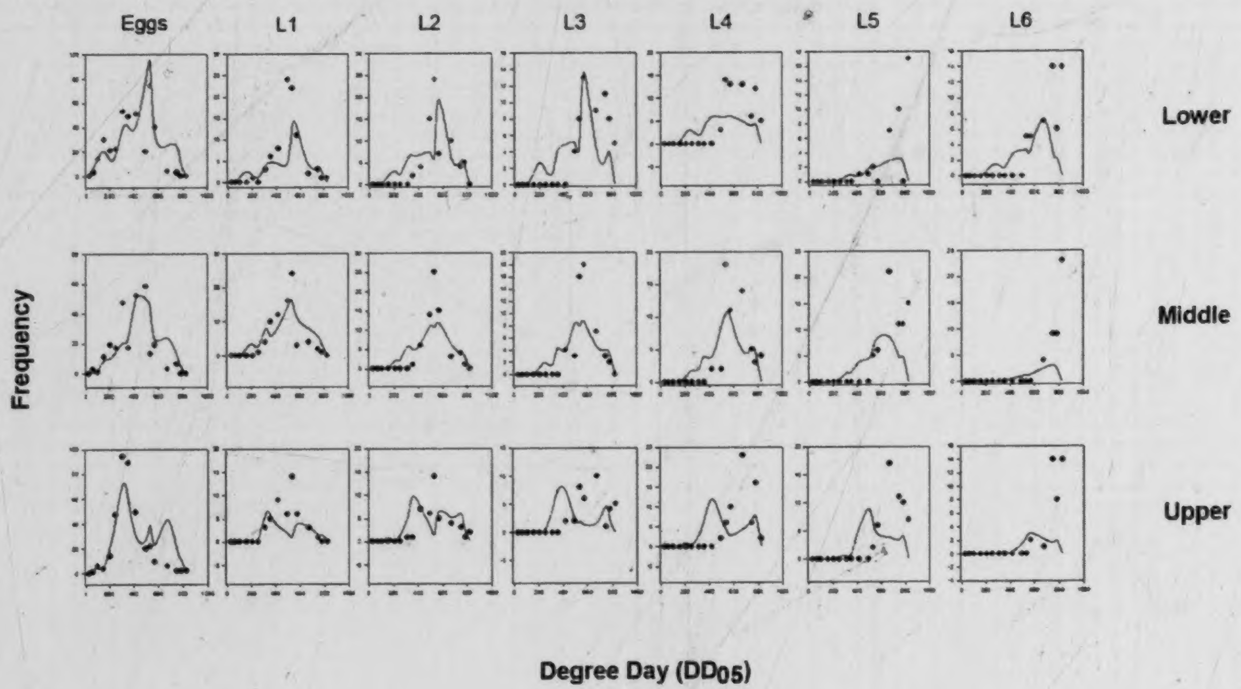
Russian Jack Park 2003



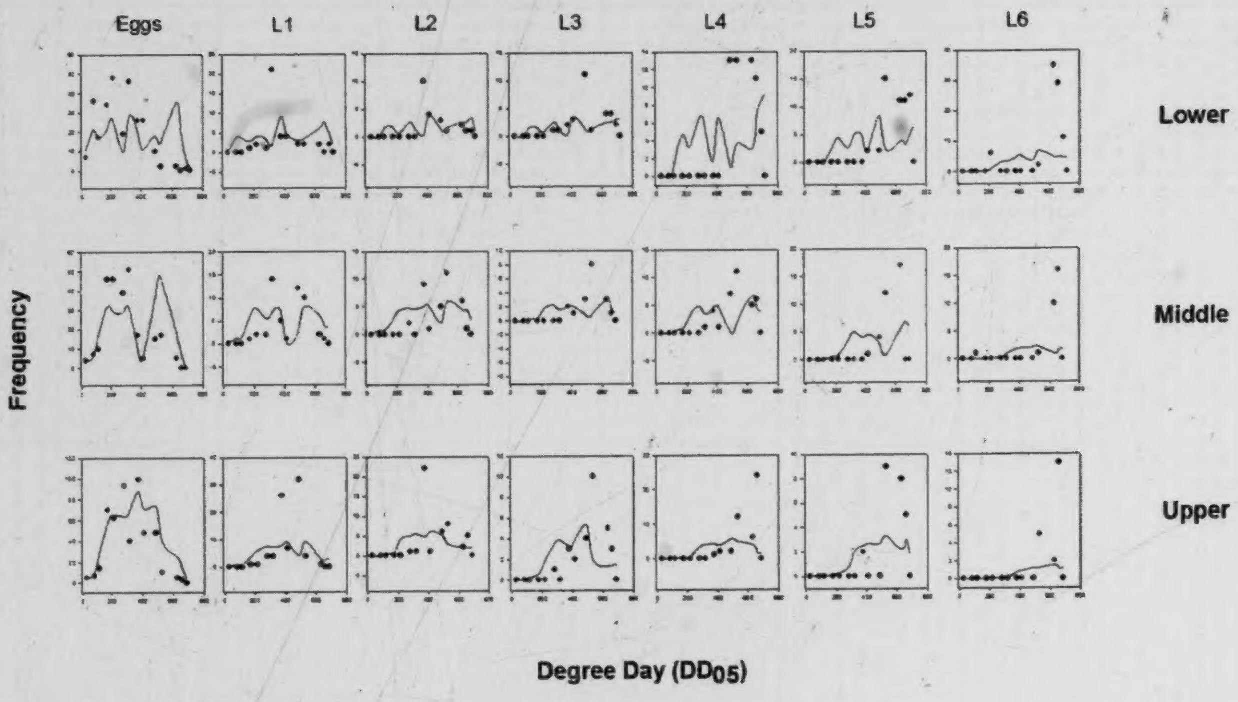
Russian Jack Park 2004



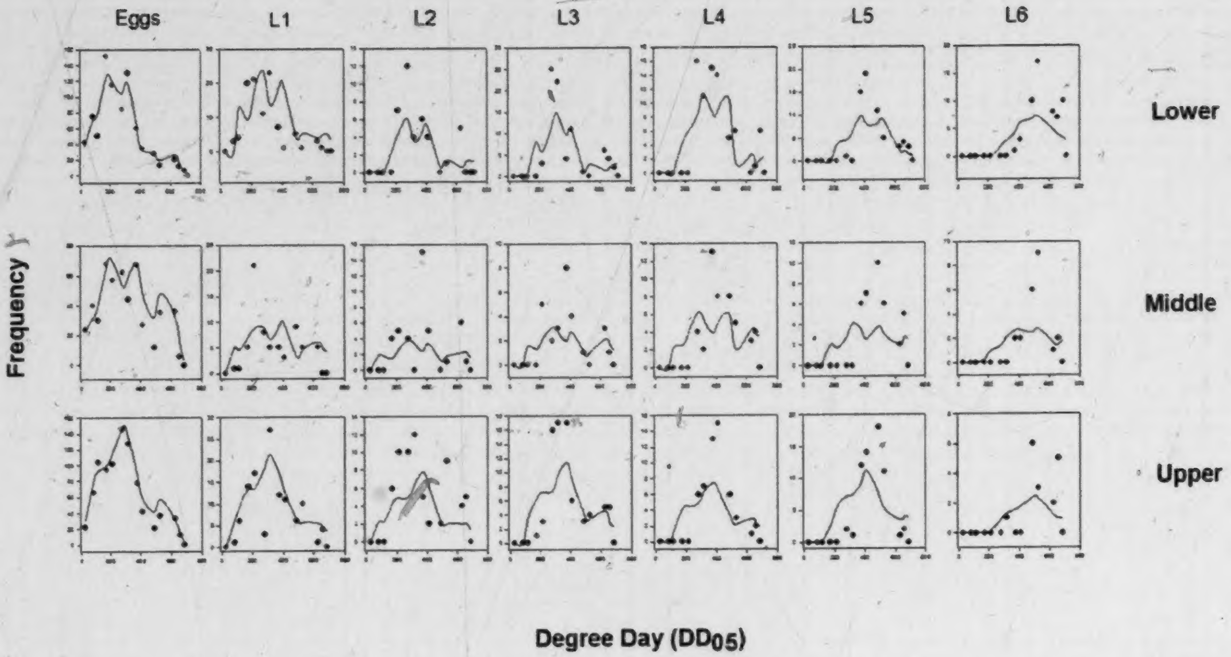
Russian Jack Park 2005



Westchester Lagoon 2003



Harvard Road (Water Tower) 2003



Appendix 4. Summary of *Profenusa thomsoni* and *Lathrolestes luteolator* rearing experiments

This appendix describes rearing experiments conducted in Anchorage, Alaska and Edmonton, Alberta.

Effect of forest type on leafminer overwintering survivorship

Background

Observations of leafminer populations in and around Anchorage, Alaska suggested *Profenusa thomsoni* populations were smaller in native forests than in urban forests. Previous studies have shown insects that overwinter in the soil such as *P. thomsoni* can suffer increased predation in native forests compared to urban forests (Liebhold *et al.* 2005; McIntyre 2000; Shrewsbury and Raupp 2006). I hypothesized that similar processes may be operating in Alaska and designed an experiment to test this hypothesis using exclusion cages. However, vandals destroyed most of the replicates in urban forests which limited the statistical power of my experiment to show a difference between urban and native forests.

Methods

Exclusion plots were used to examine the relative effects of arthropod and vertebrate predation on overwintering *Profenusa thomsoni* in urban and native forests in Anchorage, Alaska. This experiment was established in three urban forest sites in parks (BS, JL, JV; Figure 3.1) and in two native forest sites in natural areas within the Anchorage city limits. Each urban forest site had one or two replicate sets of plots separated by at least 50 m, and each native forest site included two or three replicate sets separated by at least 100 m. Urban forest sites are described in Chapter 3. Native forest sites were mixed stands dominated by birch, but also included spruce, poplar and aspen and all were contiguous with native forests. Trees in these sites were taller than in urban sites (10–20 m) with crowns beginning at 5–10 m above ground and an under story of shrubs and herbaceous plants.

The effect of predation by vertebrate and invertebrate predators on overwintering *P. thomsoni* was tested by excluding one or both groups of predators. Total adult leafminer emergence was measured as the response variable. To allow for a meaningful comparison between treatments and forest types (as urban forests generally had larger populations of leafminers than native forests) each experimental plot was stocked with the same number of *P. thomsoni* larvae. All plots were setup before the then-current generation of leafminers commenced dropping to the ground (early August 2005). However, there was the possibility that some *P. thomsoni* were in the plots prior to setup (i.e., in extended diapause). Therefore, control plots were also established to which no *P. thomsoni* were added. Each set of plots consisted of four, 1 m × 1 m square plots individually enclosed by plastic garden edging. The edging was placed in a trench dug around each plot to the depth of mineral soil and served to prevent leafminers from exiting the plot and to prevent other insects from entering it. Hardware cloth (1 cm × 1 cm metal mesh) was placed over two of each set of plots and heavy, woven landscape fabric was placed over all the plots to prevent predation (see below) and emerging leafminer larvae from falling in. The hardware cloth and landscape fabric were secured by burying them in the trench dug for the garden edging.

Following plot establishment leaves containing L3–L5 *P. thomsoni* larvae were collected in Anchorage and reared in the laboratory until L6 emerged (Chapter 4). These L6 were then used to stock the plots. Both plots covered with hardware cloth and landscape fabric and one of the plots covered with landscape fabric each received 100 L6. The plot that did not receive larvae served as the control. Larvae were introduced to the plots via a small hole cut in the landscape fabric which was then re-sealed. The plots were left closed until leafminers had ceased emerging from the overhanging trees (late October 2005). Then all plots were opened by removing the landscape fabric except for one of the two plots in each set that were initially covered with hardware cloth. That plot was left closed and became the total exclusion treatment in which no predation was expected to occur. The other plot covered with hardware cloth was used to evaluate the impact of invertebrate predation. The completely uncovered plot allowed predation by both groups. All plots were left open until early May 2006, at which time 1 m × 1 m pyramidal emergence traps (Digweed *et al.* 2003) were placed over each plot to collect the emerging *P. thomsoni* adults. The traps were left in place until emergence was no longer observed (late July 2006).

The effect of treatment and forest type on adult leafminer emergence was tested with ANOVA using PROC GLM (SAS Institute Inc. 2000-2004). The data violated the assumption of normality of residuals and homogeneity of variance and so were transformed to $\log_{10}X+1$ before analysis. However, untransformed data are presented in comparisons below.

Results

Four of five urban sites and one of five rural sites were vandalized and some replicates were destroyed. Thus not all treatments were represented equally in the data. Analyzing those replicates still in place at the end of emergence showed more sawflies emerged in urban sites than native forest sites (Figure A4.1) but there was no difference between predator exclusion treatments and the control nor did treatments vary within site (Table A4.1)

Discussion

Little overwintering mortality of *P. thomsoni* in Anchorage could be attributed to depredation, but there were distinct differences in mortality between native and urban forests. Urban sites host fewer vertebrate and invertebrate predators (Liebhold *et al.* 2005; McIntyre 2000; Shrewsbury and Raupp 2006) and thus the lack of difference between the predation treatments within urban sites was not surprising. However if predators do limit defoliator populations in forests (Elkinton *et al.* 1996) than more leafminer adults should have emerged from the vertebrate and invertebrate exclusion treatments in these sites. Since the opposite pattern emerged, it appears that neither populations in native nor populations in urban forests in AK are limited by predation. However the lack of difference among the treatments and the control could have been caused by poor survivorship of the supplemental larvae. If these larvae failed to survive, which is indicated by the lack of difference between the treatments and controls in both forests, then all *P. thomsoni* caught were present when the plots were established. This does not diminish the finding that predation does not limit *P. thomsoni* populations, at least in urban forests, as the treatments should have prevented predation of those pupae already in the soil. Furthermore it appears that a rather large number of *P. thomsoni* (40–60 per m^2; Figure A4.1) engage in some form of extended diapause. This may have implications for the ability of control efforts or biological control agents (Chapter 4) to suppress *P. thomsoni* if such a large population is maintained in the soil every year.

Rearing protocol experiments and vital rate estimates

Background

This section describes a series of modifications to the *P. thomsoni*/*L. luteolator* rearing protocol (Chapter 4) designed to increase rearing success. Overall emergence of the first (2003) generation of *L. luteolator* and *P. thomsoni* was poorer than expected (Figure 4.1) therefore I evaluated the effect of various manipulations on rearing success during all stages of the rearing process. I also took samples to estimate the overall effect of rearing on percent parasitism and mortality. A flow-chart outlining these methods as they relate to the main protocol is provided (Figure A4.2).

Methods - Experiments

Effect of shipping season on emergence

I hypothesized that spring (May) shipments of overwintered sawflies and parasitoids inhibits development (of both species). The overwintering site in Edmonton (55 degrees North latitude) experiences spring temperatures sooner and a different light-dark cycle than the rearing site in Anchorage (61 degrees North latitude). If *P. thomsoni* or *L. luteolator* respond to either of these cues shipping in spring may disrupt their post-overwinter development. Thus, shipping parasitoids to AK in early fall (September) might increase survivorship and percent emergence. This hypothesis was tested using immature sawflies and parasitoids collected during 2004 and 2005. In both years, leafminer larvae were first released into the rearing tubs and then the tubs were assigned to two treatments. For the first treatment, five rearing tubs containing a total of 3631 *P. thomsoni* larvae were shipped by air to AK in late September and overwintered there using the same procedure employed in Canada. For the second treatment seven rearing tubs (6076 larvae) were overwintered in Edmonton (using the same procedure employed in 2003) then shipped to AK the following May. Percent emergence of insects (parasitoids and sawflies) was compared between the two treatments.

Effect of shipped stage on emergence

The act of shipping rearing tubs to AK was also hypothesized as a possible cause of reduced emergence. I tested if shipping somehow disrupted the development of overwintering sawflies and parasitoids by bringing sawfly larvae to AK and allowing them to pupate. I then compared the emergence of sawflies and parasitoids from these larvae to emergence from insects that were shipped to Alaska in pupal cells in shipping tubs. This hypothesis was tested using insects collected in 2005. Immediately after emergence from leaves, larvae were placed into 150mL Ziploc bags containing moist paper towel then held for < 48 hours at 4°C. For shipment, all larvae were packed in a small picnic cooler with gel ice packs, and flown to Anchorage as carry-on luggage aboard a commercial airliner. Upon arrival in Anchorage, larvae were placed in one of four rearing tubs (described above). Two shipments totaling 1504 *P. thomsoni* larvae were made in this manner on 6 and 14 August 2005. The percent emergence of sawflies and parasitoids from these four tubs was then compared to the emergence from tubs established in Edmonton using larvae from the same generation and collection sites.

Effect of handling on emergence

I evaluated the method of rearing parasitized *P. thomsoni* larvae as a potential cause of poor emergence. If allowing larvae to emerge into the plastic bag then placing them into the rearing tub had a negative effect on development (i.e., by excessive handling or prolonging the time between emergence and constructing a pupal cell) then insects allowed to emerge directly from leaves into the rearing tubs (without handling) should have greater survivorship and thus better emergence. This experiment was implemented using insects from the 2006 generation and utilized a method modified from van Driesche *et al.* (1997). Infested leaves were collected in the usual fashion, but instead of being placed in the mesh and plastic bag apparatus they were put on a platform of 1cm × 1cm hardware cloth suspended 2.5cm above the rearing medium in rearing tub. The tub was then placed inside a sealed plastic bag to maintain humidity. As larvae completed development they dropped directly onto the rearing medium rather than being physically removed from the plastic rearing bags and placed in the rearing tub. Each rearing tub was provided with two batches of c. 500 mines (c. 450 leaves) collected 2–3 days apart from Edmonton AB and Edson AB. Each mine contained a single larvae. Using this “Drop-in” method three tubs containing approximately 2900 *P. thomsoni* larvae were established. The percent emergence of sawflies and parasitoids from these three tubs was compared to the emergence from tubs established at the same time using the standard method with larvae from the same generation and collection sites.

Effect of rearing medium on emergence

The fourth experiment examined the hypothesis that the rearing medium does not replicate the conditions in the soil and duff where *P. thomsoni* normally overwinter. If the rearing medium is less than ideal habitat, insects that overwinter in natural conditions should have better emergence than those in the rearing tubs. This hypothesis was tested using insects from the 2006 generation. A plot of turf (0.5m × 1.5m) in a small mixed stand (c. 20 birch, poplar [*Populus balsimifera*], aspen [*Populus tremuloides*], oak [*Quercus* sp.] and crab apple [*Malus* sp.]) on the grounds of the Northern Forestry Centre in Edmonton, AB was enclosed using plastic garden edging set approximately 10 cm into the ground (about the level of mineral soil in this stand) and then covered with a 1–2cm layer of rearing medium and birch leaves. Over one 24 hour period, 2986 newly emerged larvae collected in Hay River were distributed over the entire area of the enclosure. The plot was then covered with a mesh screen to prevent bird predation and a bead of Tanglefoot (The Tanglefoot Company Grand Rapids, Michigan) applied along the top of the edging to prevent incursion by predaceous arthropods (e.g., ants [Hymenoptera: Formicidae]). In the spring of 2007 the mesh screen and plastic garden edging were removed and the turf, along with 3–5 cm of the adhering soil, was cut using a flat-mouth spade and an edging tool. The turf was then divided into roughly equal sized pieces and placed into one of six rearing tubs containing c. 5cm of dry rearing medium. These tubs were then packaged and shipped to AK in the usual fashion. For the purposes of calculating an emergence rate, each of these tubs was assumed to contain 1/6 of the total number of insects introduced to the plot. The percent emergence of sawflies and parasitoids from these six tubs was then compared to the emergence from tubs established at the same time using the standard method with larvae from the same generation and collection site.

Methods - Assessment of Mortality

Pre emergence Assessment.

Parasitism and mortality associated with overwintering and shipment were estimated using independent samples taken from individual rearing tubs. A 40–300mL volume of rearing medium was removed from 1–5 randomly chosen rearing tubs at various times after larvae were placed into the rearing tubs but before emergence began: in fall before shipment (n = 3 samples), in fall after shipment (n = 3), in spring before shipment (n = 5), and in spring after shipment (n = 2). Tubs were sampled once only and the volume of rearing medium removed was replaced with an equal volume of fresh medium. Depending on the volume of the sample and the number of pupal cells recovered either all pupal cells in a sample were collected or the first 30–50 cells found were collected. For those samples where only 30–50 pupal cells were collected the remaining, un-searched volume of rearing medium was returned to its original tub. All pupal cells recovered were dissected and their contents determined.

Post emergence Assessment.

A random selection of rearing tubs was sampled after emergence had ceased in order to determine the fate of leafminers and parasitoids that remained in the rearing medium. Two cores, each 8.2cm diameter by 7–9cm deep (the depth of rearing medium in the rearing tub) were removed from each tub, one from the centre of the tub and another from a randomly chosen point no less than 2cm from the edge of the tub or from the centre core. Cores were examined in 1cm increments and all pupal cells recovered were examined to determine the fate of the insect it contained. All rearing medium in the cores taken from the 2003 generation rearing tubs was searched, but as pupal cells were not found below 5 cm depth, only the top 5 cm of soil was examined from cores taken subsequently. Counts of empty and occupied pupal cells were summed by larval collection site within each generation and analyzed qualitatively.

Percent emergence and percent mortality determined for each rearing tub or core sample was used as the raw data for analysis using ANOVA. All ANOVA were performed in SAS v 9.0 using PROC GLM (SAS Institute Inc. 2000-2004), with single rearing tubs treated as a replicates. When necessary, data were arcsine-square root transformed to meet the assumptions of homogeneity of variance and normality of residuals but untransformed data are presented.

Results - Experiments

Effect of shipping season on emergence

Neither shipping season nor collection site had a significant effect on emergence (Table A4.2; Figure A4.3A).

Effect of shipped stage on emergence

Emergence of insects shipped to AK as larvae and placed in rearing tubs upon arrival was significantly lower than from those placed in rearing tubs in Edmonton and shipped in pupal cells (Figure A4.3B). There was no effect of collection site (Table A4.3).

Effect of handling on emergence

There was no significant difference in percent emergence from tubs created in the standard fashion to those created using the "drop-in" method (Table A4.4; Figure A4.4A)

Effect of rearing medium on emergence

There was no statistically significant difference in percent emergence between insects that overwintered in the outdoor plot (Table A4.5; Figure A4.4B)

Results - Assessment of mortality

Pre emergence Assessment.

The effects of shipping or overwintering on *L. luteolator* mortality could not be tested because only three of ten samples contained dead parasitoids. Nevertheless, in those three samples percent mortality ranged from 15.3% to 33.3% but the upper estimate is from a sample containing only three *L. luteolator* pupae. Overall mortality of *P. thomsoni* pupae was also low (mean ± SE, 5.3% ± 1.4% n = 10). There was no significant effect of shipment, or overwintering on sawfly mortality but mortality did vary by collection site (Table A4.6; Figure A4.5)

Post Emergence Assessment.

A total of 1480 empty and occupied sawfly pupal cells were recovered from the core samples taken from tubs containing the 2003 (n = 528), 2004 (n = 536) and 2005 (n = 416) generations. These specimens represent 7.0–10.0% of the original sample of *P. thomsoni* larvae placed into the tubs. On average, c. 10% of the soil volume in all rearing tubs was sampled each year. Empty *P. thomsoni* pupal cells and *L. luteolator* cocoons comprised the majority of samples for all generations. All cores contained some specimens, with one exception. No specimens were recovered from cores taken from the four tubs holding sawflies shipped as larvae to Anchorage. Most insects that failed to emerge were *P. thomsoni* eonymphs or *L. luteolator* larvae or pupae (Table A4.7). Most specimens appeared to be alive, but for those that were dead I was not able to attribute a cause of mortality, but note that mechanical damage was never observed.

Discussion

None of the experimental rearing procedures caused a significant increase in the number of emerged insects, but it was shown that shipping larvae was not an effective way to transport *P. thomsoni*. Furthermore no pupal cells were found in the tubs containing *P. thomsoni* shipped as larvae, suggesting that larvae do not (or cannot) enter the eonymph stage if they are unable to enter the soil soon after exiting their leaves.

Pre- and Post-emergence assessments

Poor parasitoid emergence was likely not caused by methods used to transport rearing tubs from Edmonton to AK as there was no significant effect of shipment on sawfly mortality and no observable effect on *L. luteolator* pupae. Furthermore, transport methods did not appear to affect the pre-diapause behaviors of either the sawfly or parasitoid because all *P. thomsoni* had constructed a pupal cell and entered the eonymph stage and all *L. luteolator* had consumed their hosts and constructed a cocoon. The fact that most un-emerged sawflies and parasitoids had not developed past the overwintering stage suggests that both species have an extended diapause, but when rearing tubs from the 2003 generation were overwintered a second time and then re-caged no emergence was observed. However, emergence after a second overwintering was observed from *P. thomsoni* and *L. luteolator* reared under similar conditions but not transported to AK (Scott Digweed pers. comm.). Extended diapause is observed in other sawflies and is likely a strategy to avoid adverse environmental conditions or escape natural enemies. However, in most species of insects with extended diapause most individuals emerge after the first overwintering period (or 'adverse season' *sensu* Danks 1987), which was not the case here. Extended diapause can be associated with both extrinsic environmental factors, such as temperature or moisture levels experienced during the pre-diapause stage, or it can be under genetic control (Danks 1987). High density has been associated with extended diapause in *Diprion pini* (L.) (Hymenoptera: Diprionidae) during an outbreak (Eichhorn 1983), which, if densities in the rearing tubs were too high, may suggest a partial explanation for the reduced emergence seen here. However, the fact that percent emergence did not respond to any of the experimental manipulations, also suggests that some aspect of the developmental of *P. thomsoni* and *L. luteolator* may also be disrupted by rearing, resulting in lower than expected emergence.

Overall rearing success was poor and the ultimate cause remains unknown, but no methodological process associated with rearing could be implicated in causing poor emergence or excessive mortality. Rather, it appears *L. luteolator* and *P. thomsoni* both react poorly to rearing. While unfortunate for this study, this observation does suggest a potential avenue of research and a method of suppressing birch leafminers. If the processes controlling this aspect of *P. thomsoni* biology could be better understood, the new knowledge could facilitate the development of control tactics that prevent leafminer emergence

Rearing cage temperature monitoring

Methods

Temperature was measured hourly at each rearing site from approximately 1 June to 1 August during 2004–2006. The following measurements were recorded from thermocouples attached to a datalogger (Hobo H8, Onset Computer Corp, Bourne, MA): air temperature outside the rearing cage (all years), air temperature inside a rearing cage (all years), air temperature inside the screen tent (2004) and temperature of the rearing-medium (2005, 2006). Also, in 2005 soil temperature in a stand of native birch approximately 100m from the rearing site was recorded using an identical data logger. I expected temperatures inside the rearing cage to be higher than air temperature outside the rearing cage. Therefore, to determine the magnitude of this difference I subtracted the daily maximum air temperature outside the rearing cage from the daily maximum air temperature inside the rearing cage. I then calculated the mean daily difference over the sampled period to determine how much warmer the rearing cage was than ambient temperature. Similar comparisons were made between air temperature inside the rearing cage and inside the screen tent and between native soil and the rearing medium.

Results

Rearing cages and rearing medium were warmer than ambient air and soil temperatures. Maximum daily temperatures inside the rearing cages between 1 June and 2 August were (mean ± SE) 4.4 ± 0.2 °C (2004 with screen tent, n = 61 days) and 10.6 ± 0.6 °C (2005 without screen tent, n = 61) warmer than outside air temperature. In 2004 the screen tent was 8.2 ± 0.7 °C (n = 61) warmer than outside air temperature and 3.8 ± 0.5 °C (n = 61) warmer than the rearing cages it enclosed. In 2005 the maximum daily temperature of the rearing medium was 22.3 ± 0.7°C or 9.4 ± .7°C (n = 55) warmer than the temperature of native soil in a nearby stand (sampled period: 24 May–16 June, 29 June–6 July, 12–29 July, missing dates owing to malfunctions of the data logger measuring native soil). Measures for 2006 are not available as the data loggers frequently malfunctioned resulting in an incomplete temperature profile.

Discussion

It is possible that higher temperatures within the rearing cage and the rearing medium may have affected normal development and reduced emergence. The maximum daily temperature observed for the rearing medium in 2005 was close to the lethal temperature observed for overwintering pupae of *H. testudinea* (Graf *et al.* 1996), but emergence did not improve in subsequent years when insects were reared at other more shaded (and presumably cooler) sites. Furthermore, moderate increases in temperature should speed-up development, causing sawflies and parasitoids to emerge earlier. Instead, *P. thomsoni* emergence in the rearing cages generally coincided with emergence of the local sawfly population. Langor *et al.* (2002) reported similar rates of emergence of *L. nigricollis* from *F. pumila* when reared under similar conditions, while Guevremont and Quednau (1978) observed high rates in some years (33.6–41.6%), but low rates in others (0.20–5.4%). It would be interesting, and perhaps instructive, to compare the relative rates of emergence from this study to other species; unfortunately, most authors do not publish the number of hosts collected or give emergence rates.

Table A4.1 ANOVA table for effect of forest type and exclusion treatment on total emergence

Source	DF	Sum of Squares	Mean Square	F	*p*
Forest type	1	15.74699251	15.74699251	54.02	<0.01
Exclusion treatment	3	0.29219126	0.09739709	0.33	0.80
For. type*Ex. treat	3	0.18186224	0.06062075	0.21	0.89
Error	28	8.16156933	0.29148462		
Total	35	24.35676508			

Table A4.2 ANOVA table for effect of collection site and shipping season on emergence

Source	DF	Sum of Squares	Mean Square	F	*p*
Site	1	0.01941098	0.01941098	0.52	0.49
Shipping season	1	0.10201340	0.10201340	2.73	0.14
Site*Shipping Season	1	0.02310637	0.02310637	0.62	0.45
Error	8	0.29853097	0.03731637		
Total	11	0.47058011			

Table A4.3 ANOVA table for effect of collection site and stage shipped on emergence

Source	DF	Sum of Squares	Mean Square	F	*p*
Site	1	0.08636186	0.08636186	1.88	0.22
Stage	1	0.33356450	0.33356450	7.25	0.04
Site*Stage	1	0.02639300	0.02639300	0.57	0.48
Error	6	0.27609236	0.04601539		
Total	9	0.74700256			

Table A4.4 ANOVA table for effect of rearing tub creation method on emergence

Source	DF	Sum of Squares	Mean Square	F	*p*
Method	1	0.00000299	0.00000299	0.00	0.99
Error	4	0.09926211	0.02481553		
Total	5	0.09926511			

Table A4.5 ANOVA table for effect of overwintering location on emergence

Source	DF	Sum of Squares	Mean Square	F	*p*
Location	1	0.07249639	0.07249639	5.43	0.05
Error	8	0.10684521	0.01335565		
Total	9	0.17934160			

Table A4.6 ANOVA table for effect of collection site, overwintering and shipping on *Profenusa thomsoni* mortality

Source	**DF**	**Sum of Squares**	**Mean Square**	**F**	***p***
Site	1	0.01252435	0.01252435	9.93	0.03
Overwintering	1	0.00107776	0.00107776	0.85	0.40
Shipping	1	00082510	0.00082510	0.65	0.46
Overwintering*shipping	1	0.00393613	0.00393613	3.12	0.14
Error	5	0.00630580			
Total	9	0.01910240			

Table A4.7 Number of *Profenusa thomsoni* and *Lathrolestes luteolator* of each developmental stage recovered from *P. thomsoni* pupal cells after emergence had ceased

Generation	**Collection site**	**Number of rearing tubs sampled**	***Profenusa thomsoni***				***Lathrolestes luteolator***			
			empty cases	Live eonymphs	Dead eonymphs	Other stages*	empty cocoons	Live larvae	Dead larvae	Other stages*
2003	Fort Smith	2	134	0	1	0	6	0	0	2
	Hay River	3	293	6	17	11	42	11	1	4
2004	Fort Smith	2	69	47	6	9	18	13	1	4
	Hay River	4	120	106	20	5	62	36	3	17
2005	Fort Smith	3	70	93	11	2	3	13	0	3
	Hay River	3	96	35	12	10	32	21	0	15

* Pupae or adults still in their pupal cell (*P. thomsoni*) or cocoon (*L. luteolator*).

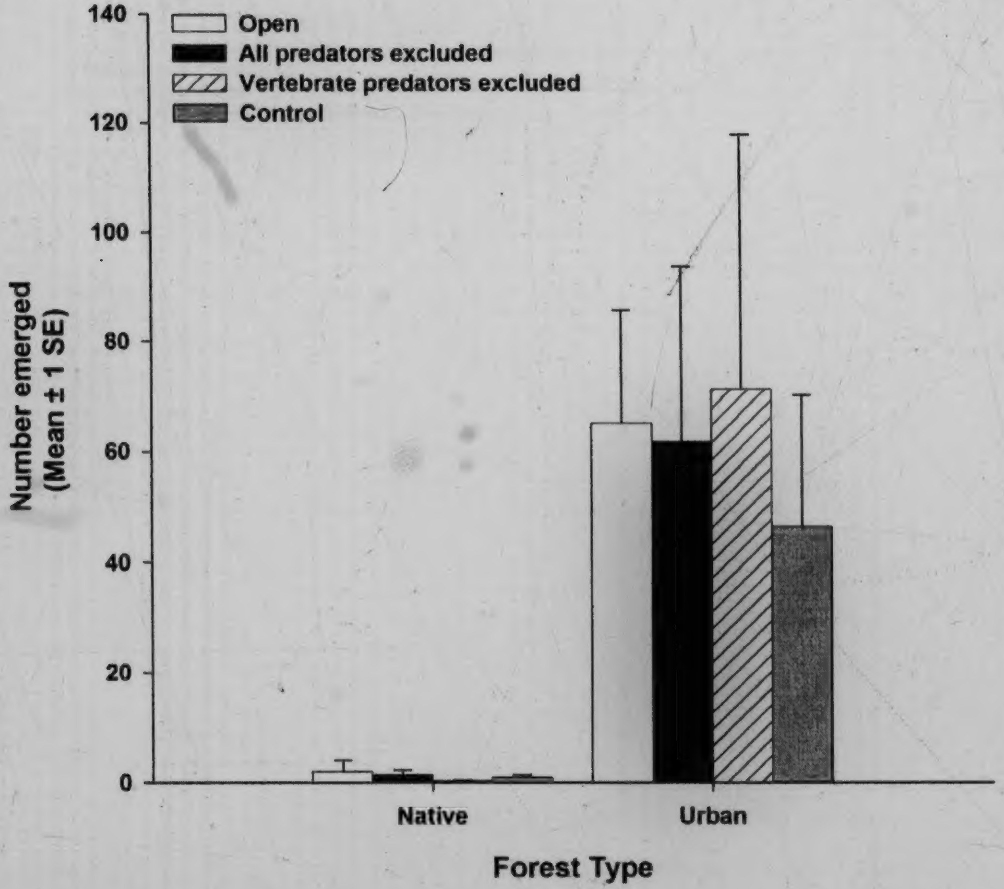
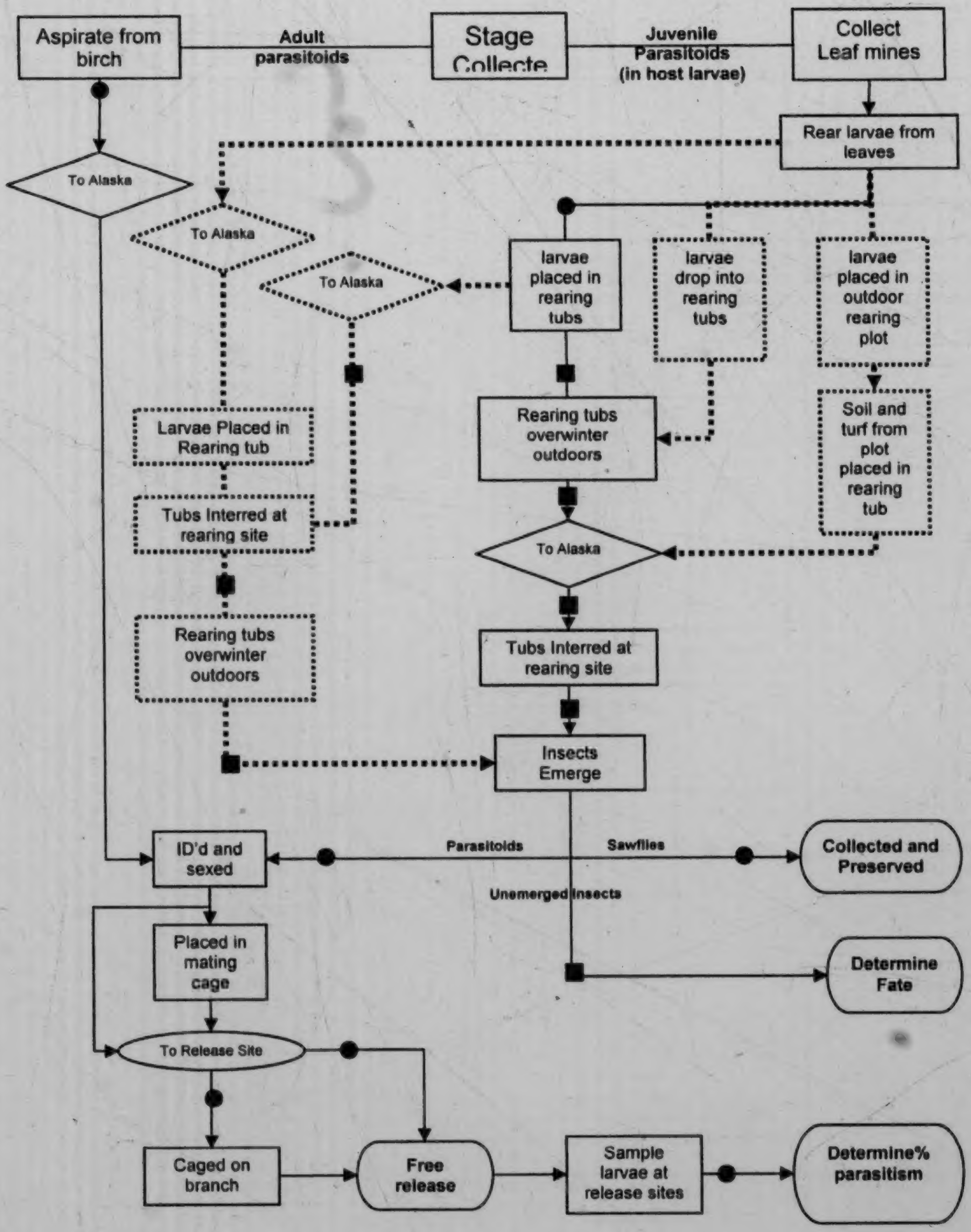


Figure A4.1 Mean number of adult *Profenusa thomsoni* collected from three predator exclusion treatments and a control in two stand types.

Figure A4.2 Graphic representation of rearing method showing important steps in the rearing process. Solid lines denote the standard method, dotted lines denote experimental methods. Boxes: Important steps, Diamonds: Transport steps, Ovals: End point for reared insects. Solid squares denote where samples of rearing medium were taken for pre- or post-emergence assessments. Solid circles indicate where counts of larvae or adults were made. See text for a more complete description of the methods


Aspirate from birch
Adult parasitoids
Stage Collecte
Juvenile Parasitoids (in host larvae)
Collect Leaf mines
Rear larvae from leaves
To Alaska
To Alaska
To Alaska
larvae placed in rearing tubs
larvae drop into rearing tubs
larvae placed in outdoor rearing plot
Larvae Placed in Rearing tub
Rearing tubs overwinter outdoors
Soil and turf from plot placed in rearing tub
Tubs Interred at rearing site
To Alaska
Rearing tubs overwinter outdoors
Tubs Interred at rearing site
Insects Emerge
ID'd and sexed
Parasitoids
Sawflies
Collected and Preserved
Unemerged insects
Placed in mating cage
Determine Fate
To Release Site
Caged on branch
Free release
Sample larvae at release sites
Determine% parasitism

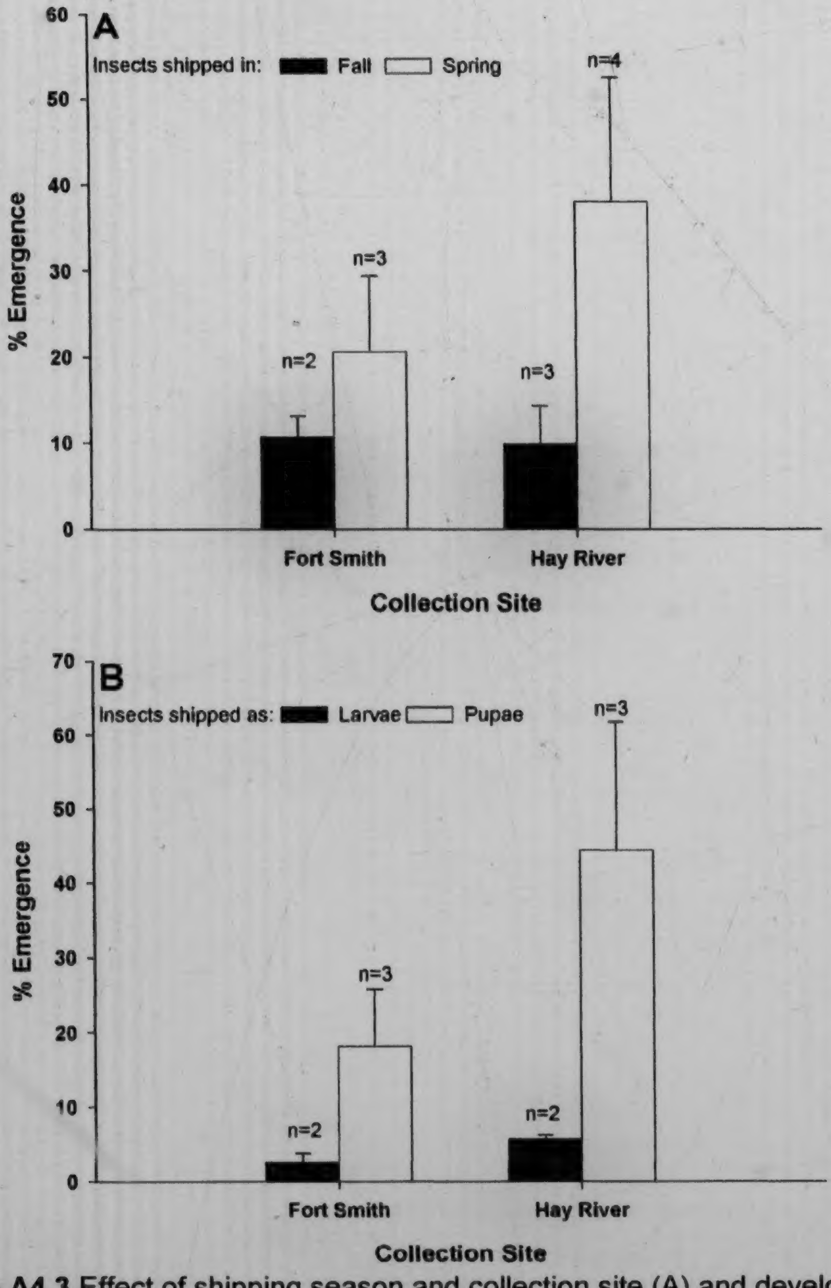


Figure A4.3 Effect of shipping season and collection site (A) and developmental stage shipped (B) on *Profenusa thomsoni* and *Lathrolestes luteolator* emergence.

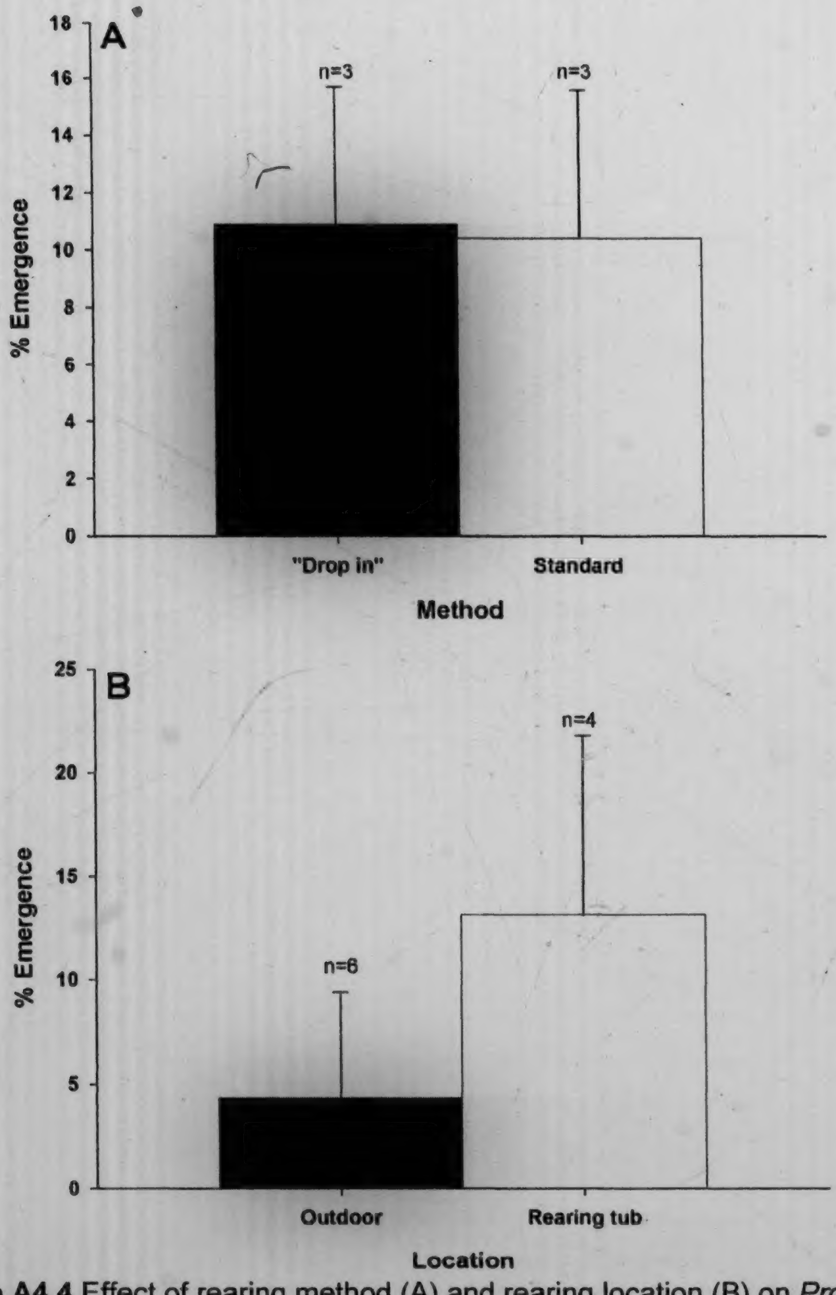


Figure A4.4 Effect of rearing method (A) and rearing location (B) on *Profenusa thomsoni* and *Lathrolestes luteolator* emergence.

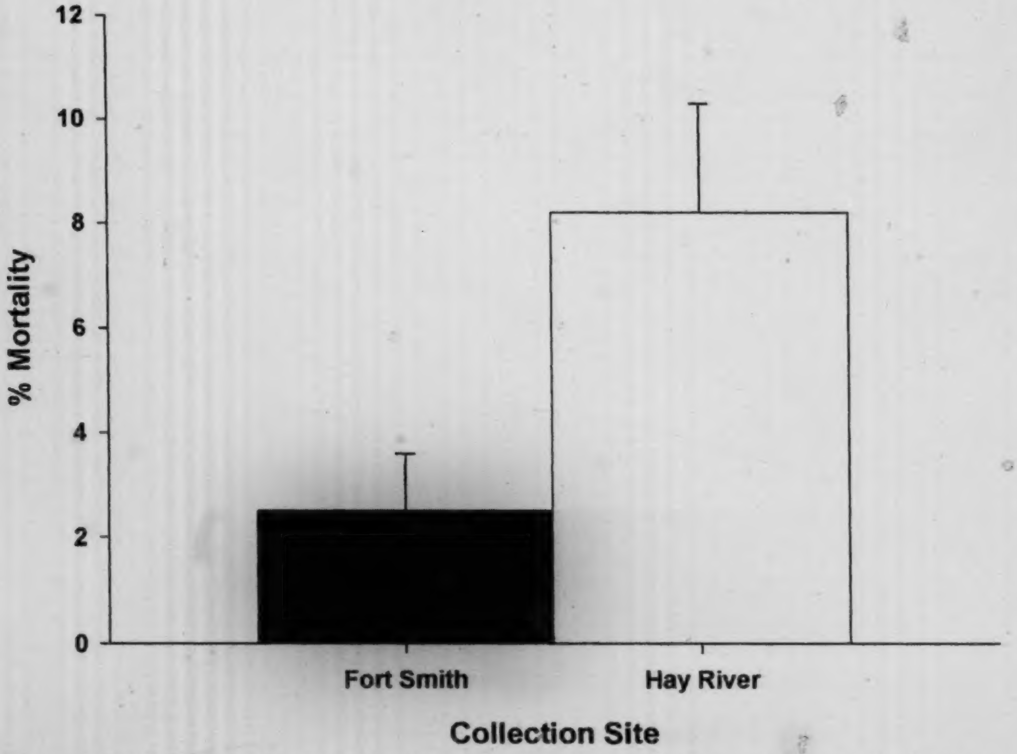


Figure A4.5 Effect of collection site on pre-emergence mortality of *Profenusa thomsoni*.

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Appendix 5. *Lathrolestes* species released in North America for biological control, 1972 - 2007.

Species	Location*					Origin	Males	Females	Total	Established†	When tested‡	Source
	Year	Cntry	St. Prov.	Name	description							
L. ensator												
	1995	CAN	QC	Freligshsburgh	Apple orchard	CHE,DEU, NLD	64	74	138	yes	4	(Vincent et al. 2001)
	1996	"	"	"	"	"	19	11	30			"
	1997	"	"	"	"	"	12	7	19			"
	1998	"	"	"	"	"	85	241	326			"
	1999	"	"	"	"	"	32	59	91			
L. luteolator												
	2001	CAN	NWT	Yellowknife	park	CAN			?[1]	not tested		(Environment Canada- Ecoaction 2003)
	2004	USA	AK	Eielson AFB	residential	"	0	2	2	unlikely		This study
	"	"	"	Anchorage[2]	park	"	15	49	64	possible	1 & 2	"
	"	CAN	NWT	Yellowknife	residential	"	50	11	61	yes	3	S Digweed unpub.
	2005	USA	AK	Anchorage	park	"	80	76	156			This study
	"	CAN	NWT	Yellowknife	residential	"	12	11	23			S Digweed unpub.
	2006	USA	AK	Anchorage	residential	"	296	122	418	yes	1	This study
	"	CAN	NWT	Yellowknife	residential	"	114	47	161			S Digweed unpub.
	2007	USA	AK	Anchorage	residential, park	"	1035	644	1679	not tested		This study
	"	"	"	Fairbanks		"	62	179	241	not tested		"
	"	"	"	Soldatna		"	40	90	130	not tested		"
	"	CAN	NWT	Yellowknife	residential	"	403	57	460			S Digweed unpub.
	"	CAN	BC	Prince George	park	"	216	38	263	not tested		CM unpub.
L. nigricollis												
	1972	CAN	NF	Pasadena	-	AUT	111	127	238	yes	2	(Raske and Jones 1975)
	1974	CAN	QC	St-Etienne-de-Lauzon	forest interior	AUT,DEU	141	273	414	yes	1	(Guèvremont and Quednau 1977, 1978)
	1975	"	"	St-Etienne-de-Lauzon	forest interior	AUT	196	304	500	yes[3]	0[4]	"

Species	Location*					Origin	Males	Females	Total	Established†	When tested‡	Source
	Year	Cntry	St. Prov.	Name	description							
L. nigricollis												
	1975	CAN	QC	Ste Foy[5]	tree nursery, park	"	102	132	234	yes	0[4]	"
	"	"	"	Route 20 nr Villeroy	roadside	"	30	28	58	no	0[4]	"
	"	"	"	St-Louis-de-Blandford	roadside	"	23	58	81	yes	0[4]	"
	1976	USA	DEL	Newark[6]	plantation	"	110	117	227	no	1 - 6	(Fuester et al. 1984)
	1977	"	DEL	Newark[6]	residential	"	34	71	105	no	1 - 7	"
	"	"	MD	Millington	river bank	"	11	25	36	no	1 - 4	"
	"	"	PA	Hickory Run	forest edge	"	197	218	415	yes	1 - 6	"
	"	"	"	Kennet Sq.	plantation	"	48	49	97	no[7]	1 - 6	"
	"	"	"	Knauertown	upland forest	"	43	45	88	no	1	"
	"	"	"	Shippensburg	upland forest	"	74	80	154	no	3	"
	"	"	"	S. Mountain	upland forest	"	19	35	54	no	3	"
	1978	"	DEL	Newark[6]	residential	AUT,FRA	54	41	95	no	1 - 5	
	"	"	PA	Wilkes-Barre	strip mine	"	609	579	1188	yes	1	"
	1979	"	DEL	Newark[6]	residential	"	260	280	540	no	1 – 4	
	"	"	NJ	Bordentown	disturbed site	"	132	141	273	no[8]	1 & 2	"
	"	"	PA	Kennet Sq.	plantation	"	971	1059	2030			"
	1980	"	PA	Philadelphia	plantation	AUT,CSK	1080	935	2015	yes	1	"
	1981	"	DEL	Newark[6]	residential	"	52	34	86	no	1 & 2	"
	"	"	NJ	Bordentown	disturbed site	"	298	406	704	no[8]	1 & 2	"
	"	"	PA	Pocono Pines	strip mine	"	599	557	1156	no	1 & 2	"
	1979	"	MA	N. Amherst	roadside	AUT,FRA	-	-	379	no[9]	1	(van Driesche et al. 1997)
	1980	"	"	Amherst[10]	roadside	"	-	-	73	no[11]	1	"
	1980	"	"	"	roadside	"	-	-	32	unlikely[12]		"

Species	Location*					Origin	Males	Females	Total	Established[†]	When tested[‡]	Source
	Year	Cntry	State/ Prov.	Name	description							
L. nigricollis												
	1989	"	"	Hadley[13]	roadside	AUT,CSK	-	-	-[14]	possible[15]	1	"
	1989	USA	RI	N. Kingstown	roadside	USA	-	-	-[14]	possible[15]	1	"
	1990	"	MA	Hadley[13]	roadside	"	-	-	-[14]			"
	"	"	RI	N. Kingstown	roadside	"	-	-	-[14]			"
	1994	CAN	AB	Edmonton[16]	tree nursery	AUT	49	54	103	yes	3-6	(Langor et al. 2000, Langor et al. 2002)
	1995	"	"	"	residential	"	315	317	652			"
	1996	"	"	"	forest edge	"	207	205	412			"

Lathrolestes ensator (Brauns) attacks *Hoplocampa testudinea* (Klug), *Lathrolestes nigricollis* (Thompson) attacks *Fenusa pumila* Leach.

* Multiple releases in one year at the same location are pooled. .

† *Yes/No* = confirmed by sampling or direct observation; *Possible* = some evidence but contradictory or anecdotal; *Unlikely* = methodological error or demonic intrusion not conducive to establishment; *Not tested* = no reported attempt to determine establishment. Establishment at a location is indicated at the first instance in the table. Some locations had multiple releases but establishment may not have occurred after the first release of parasitoids

‡ Number of years after the first release of parasitoids when tests for establishment were conducted.

Notes

[1] No adult parasitoids introduced, c. 2000 *P. thomsoni* infested leaves were transported from Edmonton to Yellowknife and deposited below birch.

[2] Three sites; 'Balto Seppla Park' (2004, 2005), 'University of Alaska-Anchorage' (2006, 2007), 'Javier de la Vega Park' (2007)

[3] Same location, different release sites in 1974 and 1975

[4] Establishment confirmed the same year by presence of parasitized second generation hosts

[5] Two sites; 'Centre de Recherches Forestières des Laurentides' & 'Base de plein air'

[6] Four sites; 'Beneficial Insects Research Laboratory' (1976, 1977), 'Campus' (1976), 'South' (1978), 'East' (1979, 1980)

[7] Parasitized hosts were recovered from this site 3 and 5 years after release but the authors were reluctant to confirm establishment

[8] Parasitized hosts were recovered at this site in 1980, 1981 but not found in 1982 or 1983.

[9] Release site destroyed during the winter after the first release

[10] Two sites; 'Harkness Rd', 'West Bay Rd'

[11] But was present in 1990, possibly an immigrant from other releases

[12] Site destroyed shortly after release

[13] Two sites; 'White', 'Rundberg'

[14] No adult parasitoids introduced, 'several thousand' host larvae collected within range of releases made by Fuester *et al.* (1987) placed into turf plots at release site

[15] Parasitoid may have already been established at this site prior to release

[16] Three sites; 'Sunstar Nurseries' (1994), 'University of Alberta' (1995), 'Howard Road' (1996)

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